UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

January 18, 2000

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Cancer Assessment Review Committee Meeting on Ziram/Ferbam

FROM: Sanjivani Diwan Sanji Diwan
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Addressees

Attached for your review is a package on Ziram\Ferbam prepared by David Nixon.

A meeting to review the carcinogenicity classification of this chemical is scheduled for Wednesday February 2, 2000 at 10:00 am in Room 813, CM2.

Addressees

K. Baetcke
L. Brunsman
W. Burnam
M. Copley
K. Dearfield
V. Dellarco
V. Dobozy
R. Hill
M. Ioannou
N. McCarroll
J. Pletcher
E. Rinde
J. Rowland
J. Stewart
C. Swentzel
L. Taylor
Y. Woo

(1)

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Ziram/Ferbam: Evaluation of Carcinogenic Potential

TO: Sanjivani Diwan, PhD
Executive Secretary, Cancer Assessment Review Committee
Health Effects Division (7509C)

FROM: David Nixon, DVM
Toxicologist, RRB4
Health Effects Division (7509C)
David Nixon 1/12/2000

THROUGH: Susan Hummel, PhD
Senior Scientist, RRB4
Health Effects Division (7509C)
Susan Hummel 1/12/2000

Attached is the Cancer Assessment Document for ziram/ferbam.

The issue of concern is the occurrence of benign hemangiomas in rats in the guideline study submitted to the Agency and other tumor incidences, C-cell thyroid tumors in male rats and lung tumors in female mice, noted in rodent studies performed by the National Toxicology Program. Also, the committee must decide whether or not it is appropriate to use the ziram database in order to evaluate the carcinogenic potential of ferbam.

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CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF *ZIRAM / FERBAM*

DRAFT REPORT

February 2, 2000

CANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

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DATA PRESENTATION: _____

DOCUMENT PREPARATION: _____

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

List the Committee members _____

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

Consulting Pathologist

Statistician Analysis

OTHER ATTENDEES:

CONTENTS

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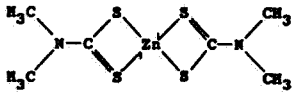
I. INTRODUCTION

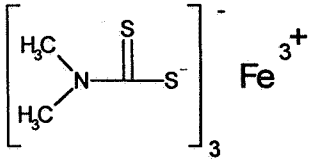
On February 9, 2000, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs is scheduled to meet to evaluate the carcinogenic potential of ziram and ferbam. This will be the first time these compounds well be assessed for carcinogenicity by the HED cancer review committee.

II. BACKGROUND INFORMATION

Ziram and ferbam are antifungal compounds used as fungicides or herbicides in agricultural and residential settings, as antimicrobials (ziram) in industrial settings, or as vertebrate repellents. The nature of its pesticidal action is unknown, but may affect fungal proteins. The PC Code of ziram is 034805 and the CAS Number is 137-30-4. The PC Code of ferbam is 034801 and the CAS Number is 14484-64-1. Ferbam has terrestrial food, non-food crop, and outdoor residential uses. Ziram has the previous listed uses plus terrestrial food + feed crop, greenhouse food crop, and indoor non-food uses.



Ziram is a white powder and has a molecular weight of 305.8, a vapor pressure ~ 10^{-7}, and a water solubility of 65 ppm. The log K_{ow} is 1.086 and the melting point is 250°C



Ferbam is a black solid and has a molecular weight of 416.5, a vapor pressure ~ 10^{-5}, a water solubility of 120 ppm, and melts with decomposition at 180°C.

Ziram and ferbam are dimethyldithiocarbamate compounds. Ziram is known to inhibit cholinesterase enzyme activity and appears to be mutagenic, although study results are conflicting. Ziram produced benign hemangiomas in the rat chronic/carcinogenicity study submitted to the Agency and no tumors in the mouse carcinogencity study. However, in a 1983 NTP report cited by IARC, ziram induced thyroid gland C-cell adenomas in male rats and lung tumors in female mice. A 1976 IARC report stated, "Ferbam has been tested by oral administration in mice and rats and by single subcutaneous injection in mice. Although no carcinogenic effect was observed in these tests, the available data are insufficient for an evaluation of the carcinogenicity of this compound to be made." Both ziram and ferbam are classified as "not classifiable as to its carcinogenicity in humans (Group 3)" by the IARC. The Agency issued a waiver in January 1991 granting the request from the registrant to allow the ziram rat and mouse carcinogenicity studies to satisfy the testing requirements for ferbam (D159794; S388171). [Attachment 1]

III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study with ziram in CD(SD)BR rats

Reference: Lindsey A.J. Powell, Sarah M. Bottomley, David Crook, Richard L. Gregson, John M. Offer, William A. Gibson, Alan Anderson (1994) Combined chronic toxicity and oncogenicity of Ziram (Technical) administered in the diet to rats. Laboratory name: Huntingdon Research Centre Ltd. Laboratory report number: ZIR 9/942098. September 27, 1994. MRID 43404201. Unpublished. [Attachment 2]

A. Experimental Design

Male and female CD(SD)BR rats, 50/sex/dose in the main group, 20/sex/dose in the satellite group were treated with Ziram (98.7%, Lot# 8331 AA) at 0, 60, 180, and 540 ppm for 104 weeks, MRID No. 43404201. These doses corresponded to achieved intakes of 0, 2.5, 7.7, and 23.7 mg/kg/day for males in the main group and 0, 3.4, 10.2, 34.6 mg/kg/day for females in the main group.

B. Discussion of Tumor Data

The dietary administration of ziram up to 540 ppm resulted in a treatment-related increased incidence of hemangiomas in mesenteric lymph nodes (5/49 or 10%), in the spleen (1/49 or 2%), and combined (6/49 or 12%) in male rats at 540 ppm. These tumors showed no evidence of malignancy. No hemangiomas were noted in the control group. Incidences of hemangiomas in historical controls ranged from 0 to 4% (mean = 1%) in lymph nodes and was 0 in the spleen [Attachment 3]. The statistical evaluation of hemangiomas in male rats revealed significant increasing trends at $p < 0.01$ and significant differences in the pair-wise comparisons of the 540-ppm group with the controls at $p < 0.05$ for mesenteric lymph node hemangiomas and for lymph node and spleen hemangiomas combined. No treatment-related tumors were identified in males in the 180- or 60-ppm groups or in females at any dosage.

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of ziram in male rats. Female rats showed a significant decreasing trend in mortality with increasing doses of ziram. [Attachment 4]

The statistical analyses of tumors in male rats were based upon Exact Trend Test and the Fisher's Exact Test for pair-wise comparisons. See Table 1 for male rat tumor analysis results. The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Table 1. Male Rats: Mesenteric Lymph Node and Spleen Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results.

ppm	0	60	180	540
mg/kg/day	**0**	**2.5**	**7.7**	**23.7**
TumorType				
Mesenteric Lymph Node Hemangiomas	0/50	0/49	0/50	5[a]/49
%	(0)	(0)	(0)	(10)
p =	0.001**	1.000	1.000	0.027*
Spleen Hemangiomas	0/50	0/49	0/49	1[b]/49
%	(0)	(0)	(0)	(2)
p =	0.249	1.000	1.000	0.495
Combined	0/50	0/49	0/49	6/49
%	(0)	(0)	(0)	(12)
p =	0.000**	1.000	1.000	0.012*

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a]First lymph node hemangioma observed at week 105, dose 540 ppm.

[b]First spleen hemangioma ovserved at week 102, dose 540 ppm.

Note: Interim sacrifice animals are not included in this analysis. There were no hemangiomas in any interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-Neoplastic Lesions

Non-neoplastic lesions are presented in Table 2. The lesions observed appear to have no relevance in regards to tumor induction. For main group males and females in the 180 and 540 ppm dose groups, there were findings of hemosiderosis in the spleen ($p<0.01$) and sinusoidal cells of the liver ($p<0.01$), bile duct hyperplasia ($p<0.05$, $p<0.01$), hyperplasia of the non-glandular epithelium of the stomach ($p<0.05$, $p<0.01$), subepithelial edema ($p<0.05$) and ulcerations ($p<0.05$) in the stomach, prominent ultimobranchial cysts in the thyroid ($p<0.01$), adipose infiltration/replacement of peripheral muscle fiber bundles ($p<0.01$), and narrowing of peripheral muscle fiber bundles ($p<0.01$) in skeletal muscle, axonal degeneration (minimal) in the spinal cord (males, $p<0.05$) [not shown in Table 10 p. 24 of DER; see p. 264 of the study], and

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axonal degeneration in the sciatic nerve (males, not statistically significant; females, $p<0.01$). In addition, the degeneration was of greater severity in treated animals than in controls and generally increased in severity with increasing dose of Ziram. There were findings for males only in the 180 and/or 540 ppm dose groups, including adipose replacement of pancreatic tissue ($p<0.05$, $p<0.01$), C-cell hyperplasia in the thyroid ($p<0.05$), hyperplasia in the parathyroids ($p<0.05$), and hypertrophy with vacuolation in the adrenal cortex ($p<0.05$). There were findings for females only in the 180 and/or 540 ppm dose group, including an increase in lipofuscin in cortical tubular epithelial cells in the kidney ($p<0.01$), acinar hyperplasia in the mammary gland ($p<0.05$) and cystic degeneration in the adrenal cortex ($p<0.01$). The findings for males in the low dose group (60 ppm) included hemosiderosis in spleen ($p<0.01$), hyperplasia of the non-glandular epithelium in the stomach ($p<0.01$), subepithelial edema in the stomach ($p<0.05$), narrowing of peripheral muscle fiber bundles in the skeletal muscle ($p<0.05$), and hypertrophy with vacuolation in the adrenal cortex ($p<0.05$). The only microscopic pathological finding occurring at a statistically significant increased incidence for females in the low dose group (60 ppm) was prominent ultimobranchial cysts in the thyroid ($p<0.05$).

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TABLE 2. MICROSCOPIC PATHOLOGY: NON-NEOPLASTIC CHANGES FOR MAIN GROUP RATS ADMINISTERED ZIRAM FOR 104 WEEKS

Pathology	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	60	180	540	0	60	180	540
No. animals (main group)	50	50	50	50	50	50	50	50
Spleen								
No abnormalities detected	22	13*	13*	14	14	9	2**	3**
Hemosiderosis	10 (2.3)[a]	22** (1.6)	29** (2.0)	23** (2.0)	24 (2.8)	29 (2.2)	38** (2.3)	39** (2.9)
Liver								
Pigment (hemosiderin) in sinusoidal cells	2 (1.5)	5 (1.2)	22** (1.3)	26** (1.4)	0 (0)	3 (1.3)	13** (1.8)	15** (1.9)
Bile duct hyperplasia	7 (1.9)	7 (2.0)	13 (2.1)	15* (2.1)	5 (1.8)	6 (1.8)	9 (2.1)	17** (2.1)
Stomach-Non-Glandular Region								
No abnormalities detected	31	28	18**	20*	32	35	27	11**
Epithelial hyperplasia	6 (2.5)	18** (2.7)	25** (2.6)	25** (2.7)	7 (2.3)	6 (2.5)	15* (2.5)	37** (2.5)
Subepithelial edema	2 (3.0)	8* (2.75)	8* (2.75)	10* (2.7)	2 (2.5)	3 (3.3)	3 (2.3)	11** (2.7)
Ulceration	5 (2.4)	8 (2.4)	14* (2.4)	14* (2.4)	3 (2.3)	5 (2.8)	6 (2.5)	12* (2.2)
Perforating ulceration, marked	0	1	0	3	0	0	0	1
Hyperplasia at the limiting ridge	1 (2.0)	2 (2.0)	3 (2.0)	3 (2.3)	1 (2.0)	2 (2.5)	4 (2.8)	1 (2.0)
Pancreas								
Replacement by adipose tissue	7 (2.4)	14 (2.4)	15* (2.3)	21** (2.7)	0/50 (0)	2/50 (1.5)	4/49 (2.0)	3/50 (2.7)
Thyroid								
No abnormalities detected	29	31	25	24	37	31	20**	17**
C-cell hyperplasia	1 (2.0)	3 (2.3)	5 (2.2)	8* (2.5)	5 (2.8)	5 (2.2)	5 (2.2)	3 (2.3)
Prominent ultimobranchial cysts	4	5	14**	15**	3	12*	22**	27**
Parathyroids								
No abnormalities detected	48/49	40*/47	44/48	39*/46	47/48	46/46	46/47	47/47
Hyperplasia	1/49 (2.0)	4/47 (2.5)	4/48 (2.3)	7*/46 (2.3)	0	0	0	0

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TABLE 2 (Continued)

Pathology	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	60	180	540	0	60	180	540
Skeletal muscle								
No abnormalities detected	38	31	13**	4**	50	43**	22**	16**
Adipose infiltration/replacement of peripheral muscle fiber bundles	5 (2.4)	10 (1.9)	27** (2.2)	43** (2.5)	0 (0)	4 (1.8)	21** (2.1)	26** (2.2)
Narrowed peripheral muscle fiber bundles	2 (2.5)	10* (1.8)	30** (2.1)	37** (2.2)	0 (0)	4 (1.8)	15** (1.7)	22** (2.0)
Spinal cord								
Axonal degeneration	15 (1.3)	23 (1.4)	20 (1.9)	22 (1.9)	13 (1.1)	10 (1.5)	15 (1.6)	13 (1.5)
Sciatic nerve								
Axonal degeneration	14 (1.6)	12 (1.5)	15 (1.7)	22 (2.0)	3 (1.0)	5 (1.2)	4 (1.3)	16** (1.4)
Kidney								
Brown pigment (lipofuscin) in cortical tubular epithelial cells	2 (1.0)	6 (1.2)	6 (1.3)	4 (1.8)	5 (1.8)	8 (1.0)	9 (1.7)	19** (1.4)
Adrenals								
Cortical hypertrophy with vacuolation	4 (2.3)	11* (2.1)	11* (2.2)	12* (2.0)	1 (1.0)	1 (2.0)	0 (0)	2 (3.0)
Cortical cystic degeneration	2 (3.0)	3 (2.0)	1 (2.0)	2 (3.5)	7 (3.6)	9 (3.2)	12 (2.9)	29** (3.4)
Lymph nodes-Cervical								
No abnormalities detected	49/50	19**/26	22**/29	42*/50	48/50	26/29	23/26	46/50
Plasmacytosis	1	5*	4	6	2	3	3	3
Ovaries								
Absence of corpora lutea	--	--	--	--	26/50	27/50	29/50	32/49
Mammary Gland								
Acinar hyperplasia	4/50 (2.3)	1/23 (2.0)	1/29 (3.0)	3/50 (2.7)	20/50 (3.0)	20/47 (2.7)	18/48 (2.6)	30*/50 (2.6)

Data adapted from Table 13, p. 142-296, MRID No. 42434001. Unless otherwise noted in the Table, the incidence of a lesion is given per 50 animals examined.

[a]Numbers in parentheses are the average severity rating of the lesion per number of affected animals, as calculated by the reviewer using the following numerical equivalents to the grade of the pathology: 1=trace, 2=minimal, 3=moderate, 4=marked, 5=severe.

*$p<0.05$

**$p<0.01$

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Organ weights were also affected by oral administration with ziram. For males, absolute organ weight for adrenals were dose-dependently decreased at weeks 52 and 104. The decrease was statistically significant at week 104 for males in the high dose group for absolute (58.8% of control, $p<0.01$) and relative (66.7% of control, $p<0.05$) adrenal weights. Relative brain weights for males in the 540-ppm dose group were statistically significantly increased at week 104 (110% of control, $p<0.05$). Relative testes weights were statistically significantly increased at week 52 for males in the 540-ppm dose group (115% of control, $p<0.05$) and at week 104 for males in the 180- (112.5% of control, $p<0.05$) and 540-ppm (127% of control, $p<0.01$) dose groups. Relative organ weights were statistically significantly increased for females in the high dose group relative to controls for brain (weeks 52 and 104, 120% of control, $p<0.01$), thyroid (week 104, 133% of control, $p<0.05$), heart (week 52, 117% of control, $p<0.01$; week 104, 112% of control, $p<0.05$), kidney (week 52, 111% of control, $p<0.05$), and adrenal (week 104, 156% of control, $p<0.01$). For females, relative liver weights were dose-dependently increased at weeks 52 and 104. The increases were statistically significant at week 52 for females in the 60-, 180-, and 540-ppm dose groups (113%, 113%, and 120% of control, respectively, $p<0.01$) and at week 104 for females in the 540-ppm dose group (112% of control, $p<0.05$). Relative pituitary weights were statistically significantly decreased (72.5% of control, $p<0.05$) at week 52 for females in the 540-ppm dose group.

D. Adequacy of the Dosing for Assessment of Carcinogenicity

Under the conditions of this study, dosing is considered adequate to assess the carcinogenic potential of ziram based on histopathology of various tissues observed in both sexes at all dosages, effects on organ weights at 540 ppm, and decreased body weight gain in males (86% of controls) and in females (74% of controls) at 540 ppm.

2. Carcinogenicity Study with ziram in CD-1 (ICR)BR mice

Reference: Lindsey A.J. Powell, Sarah M. Bottomley, David Crook, S.K. Majeed, C. Gopinath, William A. Gibson, Alan Anderson. (1994) Ziram (Technical) Potential oncogenicity to mice by repeated dietary administration for 80 weeks. Huntingdon Research Centre, Ltd., Cambridgeshire, England. Report # ZIR 12/932311, August 19, 1994. MRID # 43373701. Unpublished. [Attachment 5]

A. Experimental Design

In a 80-week oncogenicity feeding study (MRID No. 433737-01), Ziram (98.7%, Lot No. 8331 AA) was administered in the diet to 50 male and 50 female Crl: CD-1 (ICR) BR mice per group at 0, 29, 75, 225, or 675 ppm. The doses corresponded to overall mean

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doses of about 0, 3, 9, 27, and 82 mg/kg/day for males, and to 0, 4, 11, 33, and 95 mg/kg/day for females.

B. Discussion of Tumor Data

The dietary administration of ziram up to 675 ppm did not result in an overall treatment-related increase in tumor incidences in Crl:CD-1(ICR)BR mice. No statistically significant differences in mortality or indications of a treatment-related effect on the incidence or distribution of decedents were noted.

C. Non-neoplastic lesions

The incidences of roughened and white forestomach were increased in females at 675 ppm compared to controls (14.3% compared to 2.6% in controls for roughened stomach, 20.0% compared to 7.9% in controls for white stomach), but not in males at study termination. Irregular cortical scarring of the kidneys was seen in 42.4% of males at 675 ppm compared to 14.7% in controls at study termination. The incidence of brown discoloration of the kidneys was also slightly increased (9.1%) at 675 ppm compared to controls (0) in males.

Significant hepatocyte enlargement occurred in all treated groups especially in the animals that completed the study. However, the incidence of hepatocyte enlargement tended to peak in the middle dose groups and decrease at the high dose. Also, most incidences of hepatocyte enlargement were graded as minimal throughout all dose groups. These effects are most likely due to an adaptive response by the liver to the test substance. Urinary bladder epithelial hyperplasia increased in a dose-related manner from total control incidences of 14.0% in males and 0 in females to incidences of 62.0% in males and 28.6% in females at the high dose. The increased incidence of urinary bladder epithelial hyperplasia was not as great in females as in males; however, the overall incidence of bladder epithelial hypertrophy increased from 12.5% in the controls to 28.6% at the high dose in females compared to no increase in males. No dose-related microscopic pathologies were seen in the stomach or kidneys that would correspond to the changes observed on gross examination.

D. Adequacy of Dosing for Assessment of Carcinogenicity

The doses were found to be adequate to test its carcinogenic potential based on increased incidences of urinary bladder epithelial cell hyperplasia in males at 225 and 675 ppm and in females at 675 ppm, increased incidences of urinary bladder epithelial cell hypertrophy in females at 675 ppm, decreased absolute brain weight in males at 225 and 675 ppm, and decreased body weight gain in males at 225 ppm (77% of control) and 675 ppm (56% of controls) and in females at 675 ppm (80% of controls).

3. National Toxicology Program Two-year Carcinogenicity Study with ziram in F344/N rats.

Reference: U.S. National Toxicology Program (1983) Carcinogenesis Bioassay of Ziram (Cas No. 137-30-4) in F344/N Rats and B6C3F$_1$ Mice (Feed Study)(NTP Technical report series No. 238), Research Triangle Park, NC. [Attachment 6]

A. Experimental Design

In a 2-year carcinogenicity feeding study, Ziram (89% pure, with 6.5% thiram) was administered in the diet to 50 male and 50 female F344/N rats per group at 0, 300, or 600 ppm. The doses corresponded to overall mean doses of about 0,11, or 22 mg/kg/day for males and to 0, 13, or 26 mg/kg/day for females.

B. Discussion of Tumor Data

C-cell carcinomas of the thyroid in male rats occurred with a statistically significant positive trend ($p<0.01$) and the incidence in the 600-ppm group was significantly higher ($p<0.05$) than that in the controls (control, 0/50, 0%; 300 ppm, 2/49, 4%; 600 ppm, 7/49, 14%). The incidence in the high-dose group also exceeded historical control incidences from the same laboratory (18/584, 3%; range 0% to 8%). The combined incidence of males with either C-cell adenoma or carcinoma also showed a statistically significant ($p<0.05$) positive trend (control, 4/50, 8%; 300 ppm, 9/49, 18%; 600 ppm, 12/49, 24%). There were no significant histopathologic changes noted in the follicular cells.

Survival of rats of each sex were not adversely affected by ziram.

The statistical analyses of tumors in male rats were based upon the Cochran-Armitage Trend Test and the Fisher's Exact Test for pair-wise comparisons. See Table 3 for male rat tumor analysis results.

NTP concluded that ziram was carcinogenic for male F344/N rats, causing increased incidences of C-cell carcinomas of the thyroid gland, but not carcinogenic for female F344/N rats.

Table 3. Male Rats: Thyroid C-cell Tumor Rates and Cochran-Armitage Trend Test and Fisher's Exact Test Results.

ppm	0	300	600
mg/kg/day	**0**	**11**	**22**
TumorType			
Thyroid C-Cell Adenoma % p =	4/50 (8) 0.422	7/49 (14) 0.251	5/49 (10) 0.487
Thyroid C-Cell Carcinoma % p =	0/50 (0) 0.003**	2/49 (4) 0.242	7/49 (14) 0.006**
Combined % p =	4/50 (8) 0.020*	9/49 (18) 0.109	12/49 (24) 0.024*

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-neoplastic lesions

C-cell hyperplasia in the thyroid gland was noted in males in the control and all dose groups, but did not appear to be dose related (control, 7/50, 14%; 300 ppm, 12/49, 24%; 600 ppm, 11/49, 22%). No treatment-related effects on C-cell histopathology were noted in females.

D. Adequacy of Dosing for Assessment of Carcinogenicity

Since there were no treatment-related effects on mortality, clinical signs, body weight, or food consumption, and minimal non-neoplastic histopathology, NTP concluded that the amount of dosing may not have been adequate and rats of both sexes may have tolerated higher doses. Dosing was selected based on decreased mean body weight gain in a 13-week study.

4. National Toxicology Program Two-year Carcinogenicity Study with ziram in B6C3F$_1$ mice.

Reference: U.S. National Toxicology Program (1983) Carcinogenesis Bioassay of Ziram (Cas No. 137-30-4) in F344/N Rats and B6C3F$_1$ Mice (Feed Study)(NTP Technical report series No. 238), Research Triangle Park, NC. [Attachment 7]

A. Experimental Design

In a 2-year carcinogenicity feeding study, Ziram (89% pure, with 6.5% thiram) was administered in the diet to 50 male and 50 female B6C3F$_1$ mice per group at 0, 600, or 1200 ppm. The doses corresponded to overall mean doses of about 22 or 196 mg/kg/day for males and to 0, 131, or 248 mg/kg/day for females.

B. Discussion of Tumor Data

Alveolar/bronchiolar adenomas of the lung in female mice occurred with a statistically significant positive trend ($p<0.01$) and the incidence in the 1200-ppm group was significantly higher ($p<0.05$) than that in the controls (control, 2/50, 4%; 600 ppm, 5/49, 10%; 1200 ppm, 10/50, 20%). Combined alveolar/bronchiolar adenomas or carcinomas in female mice occurred with a statistically significant positive trend ($p<0.05$) and the incidence in the 1200-ppm group was significantly higher ($p<0.05$) than that in the controls (control, 4/50, 8%; 600 ppm, 19/50, 38%; 1200 ppm, 16/49, 33%). The incidences of alveolar/bronchiolar adenomas and combined adenomas or carcinomas in female mice at 1200 ppm exceeded the range for the historical controls also. Historical control data on alveolar/bronchiolar adenomas show an incidence of 18/501 (3.6%) from the same laboratory and 134/2788 (4.8%) in female mouse controls across the Bioassay Program with a range of 0/50 to 7/50 (14%). The combined incidence of alveolar/bronchiolar adenomas or carcinomas in control females is 25/501 (5.0%) from the same laboratory and 184/2788 (6.6%) with a range of 0/50 to 8/50 (16%) across the Bioassay Program.

Survival of mice of each sex were not adversely affected by ziram.

The statistical analyses of tumors in female mice were based upon the Cochran-Armitage Trend Test and the Fisher's Exact Test for pair-wise comparisons. See Table 4 for female mouse tumor analysis results.

NTP concluded that oral administration of ziram to female B6C3F$_1$ mice resulted in increased incidences of alveolar/bronchiolar adenomas and of combined alveolar/bronchiolar adenomas or carcinomas. The interpretation of this increase in lung tumors, however, was complicated by an intercurrent Sendai virus infection.

Table 4. Female Mice: Lung Alveolar/Bronchiolar Tumor Rates and Cochran-Armitage Trend Test and Fisher's Exact Test Results.

ppm	0	600	1200
mg/kg/day	**0**	**131**	**248**
TumorType			
Lung Alveolar/ Bronchiolar Adenoma	2/50	5/49	10/50
%	(4)	(10)	(20)
p =	0.009**	0.210	0.014*
Lung Alveolar/ Bronchiolar Combined Adenoma/ Carcinoma	4/50	6/49	11/50
%	(8)	(12)	(22)
p =	0.031*	0.357	0.045*

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.
Incidences of carcinoma alone were not reported.

C. Non-neoplastic lesions

Alveolar epithelium hyperplasia in the lungs was noted in females in the control group and both dose groups and was dose-related (control, 2/50, 4%; 600 ppm, 4/49, 8%; 1200 ppm, 10/50, 20%). Alveolar epithelium hyperplasia in the lungs was not noted in males at any dosage level. Pulmonary adenomatous hyperplasia was noted in control and dosed males (control, 15/49, 8%; 600 ppm, 19/50, 38%; 1200 ppm, 16/49, 33%) and in control and dosed females (control, 18/50, 36%; 600 ppm, 27/49, 55%; 1200 ppm, 26/50, 52%). This particular histopathological finding is consistent with chronic Sendai virus infection which was confirmed by serology performed on untreated animals housed in the same room and from the same shipment. Six of the 26 1200-ppm group females with the adenomatous hyperplasia had pulmonary tumors, whereas four of the 24 1200-ppm group females without pulmonary adenomatous hyperplasia had pulmonary tumors also. One of 27 600-ppm group females with adenomatous hyperplasia had a pulmonary tumor.

D. Adequacy of Dosing for Assessment of Carcinogenicity

Dosing wass considered adequate based on decreases in mean body weight gain in males (both doses: 10-25% decrease compared to controls) and in females (1200 ppm: 13-23% decrease after day 80 compared to controls), decreased food consumption at 1200 ppm in males (78% of control) and in females (85% of control), and pulmonary histopathology findings. Dosing was selected based on decreased mean body weight gain (26% or more decrease compared to the control) in males and females receiving 2500 or 5000 ppm in a 13-week study.

IV. TOXICOLOGY

1. Metabolism

Groups of 15 male and 15 female rats were administered Ziram/14C-Ziram by gavage at doses of 15 mg/kg (Group 2, single low dose), 15 mg/kg/day for 14 days followed by a single dose of radiolabeled Ziram (Group 3), or 352 mg/kg (Group 4, single high dose). Controls (Group 1) received only the methylcellulose vehicle. Radioactivity excreted in the urine and feces was monitored for 168 hours (single low dose, multiple low dose, and single high-dose), and expired air for all three dose groups was monitored for 96 hours. Additionally, radioactivity in tissues and carcass were measured (MRID 42391001).

Groups of 15 male and 15 female rats were administered Ziram/^{14}C-Ziram by gavage at doses of 15 mg/kg (Group 2, single low dose), 15 mg/kg/day for 14 days followed by a single dose of radiolabeled Ziram (Group 3), or 352 mg/kg (Group 4, single high dose). Controls (Group 1) received only the methylcellulose vehicle. Radioactivity excreted in the urine and feces was monitored for 168 hours (single low dose, multiple low dose, and single high-dose), and expired air for all three dose groups was monitored for 96 hours. Additionally, radioactivity in tissues and carcass were measured.

No studies identifying the metabolites of ziram were available.

2. Mutagenicity:

Six genetic toxicology studies on Ziram have been submitted. Studies from the National Toxicology Program (NTP) and from the open literature were also available for review. The findings indicate that Ziram causes base-pair substitutions in DNA-repair deficient *Salmonella typhimurium* TA1535 and TA100 and *Escherichia coli* WP2 uvrA but not in the strains (*S. typhimurium* TA102 and TA104) that show specificity for oxidative damaging agents. In general, the response in bacteria was obtained in both the presence and absence of S9 activation. Ziram produced mixed results for gene mutations in mouse lymphoma cells and was not active for forward gene mutations in Chinese hamster V79 cells. Conflicting results were also seen in the in vitro cytogenetic assays but the

preponderance of assays favor a positive response at noncytotoxic doses. Nevertheless, the induction of unstable chromosome aberrations by Ziram cast doubts on the relevance of this finding as an influence on the initiation of carcinogenesis. Ziram was also found to be negative for unscheduled DNA synthesis (UDS) both in vitro and in vivo. The in vivo data from the open literature suggest that Ziram is not clastogenic or aneugenic in mice. While there is evidence from an article, which provided very limited data, of dominant lethal mutations in two mouse strains and alteration of sperm morphology in mice, these findings should be viewed with caution since Ziram did not cause infertility in a two-generation rat reproductive toxicity study, was not shown to be a developmental toxicant in rats but did produce equivocal evidence of malformations in rabbits.

Conflicting mutagenicity as well as carcinogenicity and reproductive/developmental results were also obtained for other members of the dimethyldithiocarbamate class of compounds such as thiram, ferbam, the sodium and potassium salts of dimethyldithiocarbamate and lead dimethyldithiocarbamate. The only consistent finding among the studied members of this chemical class was a direct mutagenic effect on the base-pair substitution strains of *S. typhimurium*. Therefore, until a plausible explanation is obtained for the disparate results and, in view of the mutagenic effects in bacteria, the weight-of-the-evidence indicates that a mutagenic mode of action can not be ruled out for Ziram. Studies supporting these conclusions are presented below:

GENE MUTATIONS

1) *Salmonella typhimurium*/ mammalian microsome gene mutation assay: The assay was positive with dose-related and reproducible ≈≥2-fold increases in mutant colonies of strain TA100 at 66.7-333.3 μg/plate without S9 activation or 33.3-333.3 μg/plate +S9. Greater than 2-fold increases in mutant colonies were also seen for strain TA1535 at 66.6 and 100 μg/plate +S9. The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a bacterial gene mutation assay (MRID No. 00147462).

2) *S. typhimurium*/ mammalian microsome gene mutation assay: Independent trials were positive with dose-related and reproducible ≥2-fold increases in mutant colonies of strain TA100 at 50, 75 and 100 μg/plate but only in the presence of 20-30% S9 in the cofactor mix. The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a bacterial gene mutation assay (MRID No. 41642901).

3) *S. typhimurium*/ mammalian microsome gene mutation assay: Ziram was one of 250 coded compounds evaluated in NTP's collaborative mutagenicity screening project of the S. typhimurium/ mammalian microsome gene mutation assay. Results were positive for strain TA100 at 10-333 μg/plate without and with S9 derived from Aroclor 1254-induced rat livers and at 33-333 μg/plate with hamster livers. Increases approaching or greater than 2-fold were also seen for strain TA1535 at 100 μg/plate with rat liver S9 or at 33-333 μg/plate with hamster liver S9. The study is classified as Acceptable and

satisfies the requirements for FIFRA Test Guideline 84-2 for a bacterial gene mutation assay (Haworth et al., 1983).

4) In vitro mammalian cell forward gene mutation assay in mouse lymphoma L5178Y cells: As part of the NTP evaluation of this mammalian cell test system, Ziram was found to be positive for the induction of gene mutations at all assayed doses in Trial 1 (0.625-1.0 μg/mL) and in Trial 2 (0.1-1.8 μg/mL). Relative total growth was 18% at 1.0 μg/mL or 8% at 1.4 μg/mL; lethality was seen at levels $\geq$1.8 μg/mL. The test was conducted only in the absence of S9 activation and colony sizing was not performed. The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a mammalian cell gene mutation assay (McGregor et al., 1988).

CHROMOSOME ABERRATIONS

5) In vitro mammalian cell cytogenetic assay in Chinese hamster ovary (CHO) cells: The test was negative up cytotoxic levels (, doses that caused a $\geq$50% reduction in the mitotic index) (0.025μg/mL -S9 or 1 µg/mL + S9). The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for an in vitro mammalian cell cytogenetic assay (MRID 41287802).

6) In vitro mammalian cell cytogenetic assay in CHO cells: In contrast to the above negative results in CHO cells, the NTP-sponsored evaluation of Ziram indicated significant and reproducible increases in structural chromosome aberrations at 0.025 and 0.05 μg/mL -S9 or 1.5 and 1.75 μg/mL +S9. In all trials, increases in simple chromatid or chromosome aberrations (e.g., breaks, fragments and double minutes) and complex aberrations (e.g., interchanges and rearrangements) with a preponderance of simple aberrations was reported. There was, however, no reproducible induction of sister chromatid exchanges (SCE) at 0.001-0.025 μg/mL -S9 or 0.16-1.75 μg/mL +S9. The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for in vitro cytogenetic mutagenicity data (Gulati 1989).

OTHER MUTAGENIC MECHANISMS

7) In vitro unscheduled DNA synthesis (UDS) in primary rat hepatocytes: Independent trials were negative up to the highest dose tested (1.0 μg/mL). The study is currently classified as Unacceptable because the highest dose tested did not cause toxicity. However, a reexamination of the data show clear evidence of cytotoxicity at higher doses ($\geq$3.16 μg/mL). Accordingly, the study should be reclassified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for an in vitro UDS assay (MRID No. 41287801).

INFORMATION FROM THE OPEN LITERATURE

Prokaryotic Test Systems

In agreement with the findings from the submitted assays and the NTP-sponsored study, Ziram induced reverse gene mutations in *S. typhimurium* strain TA100 (Moriya et al., 1976; Franekic et al., 1994; Tinkler et al., 1998; and Crebelli et al., 1992). Mutagenesis was seen in both the presence and absence of S9 activation by all of these authors. Furthermore, Franekic et al. (1994), Tinkler et al. (1998) and Crebelli et al. (1992) reported positive results in *S. typhimurium* strain TA1535.

Crebelli et al. (1992) also reported that Ziram induced a mutagenic effect in *Escherichia coli* WP2 uvrA both with and without S9 activation but was negative in *E. coli* WP2 and *S. typhimurium* strain TA102. The negative results with *S. typhimurium* strain TA102 were confirmed by Franekic et al., (1994) who also found that Ziram tested negative with *S. typhimurium* strain TA104. It is of note that both of these Salmonella strains were specifically developed by Levin et al. (1982) to detect oxidative mutagens. The positive results in *S. typhimurium* TA100 and TA1535 and in *E. coli* WP2 uvrA coupled with the negative findings for *S. typhimurium* TA102 and TA104, as well as *E. coli* WP2 suggests that the mutations induced by Ziram do not operate through oxidative damage since the mutagenic profile of oxidative agents shows preferential activity toward DNA repair-proficient strains such as *S. typhimurium* TA102 and TA104 and *E. coli* WP2. The lack of a positive effect in *S. typhimurium* TA102 or TA104 is in direct conflict with Rannung and Rannug's (1984) argument that the mechanism for dimethyldithiocarbamate mutagenicity in bacteria is associated with oxidative stress.

Despite the clear evidence of mutagenicity in bacteria, Ziram did not alkylate the acellular nucleophiles (4-p-nitrobenzyl)-pyridione or deoxyguanosine (Hemminiki et al., 1980). These findings (, positive for mutagenicity in Salmonella but negative for electrophilicity) are consistent with Ashby's and Tennant's listing of Ziram as a positive mutagen in Salmonella and as a "non-alerting" carcinogen affecting a single species, sex and site. Franekic et al. (1994) listed Ziram as the most potent bacterial mutagen not requiring S9 activation among the dimethyldithiocarbamates (thiram, zineb S-65 and ethylenethiourea) that were tested but considered Ziram to be negative for mitotic chromosome malsegregation in Saccharomyces cerevisiae D6.1M.

Eukaryotic Test Systems

Data from the mouse lymphoma assays with Ziram produced conflicting results; it was reproducibly positive in the NTP study but yielded negative and/or inconclusive findings in the study of Tinkler et al.,(1998). In the latter study, negative results were obtained without S9 activation and Ziram was considered equivocal in the presence of S9 at doses that reduced cell survival to $\leq$15% of control. It has also been reported to be negative for gene mutations in Chinese hamster V79 cells (Donner et al.,1983). Similarly, the negative findings from the submitted in vitro cytogenetic assay in CHO cells neither

agree with the data from the NTP study that used the same cell line nor with the dose-related and significant increases in structural chromosome aberrations in Chinese hamster epithelial liver (CHEL) and in CHO cells reported by Mosesso et al. (1994). In the study of Mosesso et al., significant and dose-related increases in the yield of cells with structural chromosome aberrations were seen at 0.22-1.00 μg/mL +S9 in CHEL cells or 1.0 or 2.15 μg/mL +S9 in CHO cells. Under both test systems, the major types of aberrations scored at noncytotoxic doses (i.e, mitotic indices were $\geq$85% of control for CHEL cells and $\geq$61% of control for CHO cells) were chromatid breaks and chromatid and chromosome exchanges. Tinkler et al., (1998) also reported a positive and reproducible dose-related clastogenic response in cultured human lymphocytes at 10-15 μg/mL+S9. Although the type of aberrations were not reported, the investigator did state that the clastogenic activity of Ziram was not associated with excessive cytotoxicity as indicated by the mitotic indices, which ranged from 64 to >100% of control at 10 μg/mL to 44% of control at 15 μg/mL. In all studies reporting positive in vitro clastogenesis; however, most of the gross structural damage to the chromosomes (chromatid and chromosome breaks and exchanges) can be classified as unstable and would likely lead to cell death. Hence, the relevance of the positive cytogenetic assays to a direct mutagenic mode of action for Ziram is not certain. Ziram was also shown to induce metaphase arrest (c-mitosis), multipolarity and anaphase disturbances as well as chromosomal aberrations such as micronuclei, bridges and polyploidy in Allium ascalonicum (Franekic et al., 1994). The study authors concluded that the evidence of spindle dysfunction, metaphase arrest and micronuclei induction was suggestive of aneuploidy. No other data suggesting that Ziram induces aneuploidy were found.

No in vivo studies were submitted by the registrant. However, in the adult male feeding *Drosophila melanogaster* mutagenicity tests sponsored by NTP, Foureman et al. (1994) observed that Ziram induced sex-linked recessive lethal mutations but not reciprocal translocations. Although additional positive results have been reported in the sex-linked recessive lethal and the somatic and germinal mosaic assays in *D. melanogaster* (Hemavathi et al., 1989), the studies were performed with Cuman L, a formulation containing only 27% Ziram (other components were not specified). Crebelli et al., (1992) indicated that the significant induction of micronucleated polychromatic erythrocytes (MPCEs) seen in bone marrow cells harvested from male B6C3F1 mice 24 hours after the intraperitoneal administration of the mid-dose (5 mg/kg Ziram, 98.5%) was inconclusive because the effect was confined to this sex, dose and sample time. No other in vivo cytogenetic assays with somatic cells were found in the open literature.

In contrast, evidence of infertility, pathology and chromosome aberrations in testicular cells and embryonic deaths, dominant lethal mutations and skeletal malformations were reported in the C3H and AK mouse strains by Cilievici et al. (1983). However, these unconfirmed findings should be viewed with caution because very limited data and study details were provided, the purity of the test substance was not specified, and the sample size was inadequate. In addition, the data indicating germinal cell effects were not supported by the two-generation reproductive study (MRID No. 43935801); there was no evidence in this study of increased infertility or embrotoxicity. Ziram did, however,

produce equivocal evidence of malformations in rabbits (MRID No. 00161316) but not in rats (MRID 41908701). Nevertheless, Hemavathi et al. (1993), demonstrated sperm abnormalities in Swiss albino mice receiving intraperitoneal administrations of 50 or 100 mg/kg (single dose) or 25 mg/kg Ziram (purity not specified) once daily for 5 days. While the induction of spermhead abnormalities may not be related to genetic damage in the exposed male, these findings do show that Ziram or its metabolites are capable of reaching the testes.

Ziram tested positive for DNA damage in DNA-repair deficient *Bacillus subtilis* M45 (rec-) as compared to the DNA-repair proficient strain, H17 (rec+) (Shirasu et al., 1976) but was negative for UDS in cultured rat hepatocytes (MRID No. 41287801), in hepatocytes harvested from rats pretreated with either Aroclor 1254 or 3-methylcholanthrene (Shaddock et al., 1990) or following in vivo exposure (Tinkler et al., 1998).

3. Structure-Activity Relationship

Ziram is a member of the dimethyldithiocarbamate class of compounds, which includes Thiram, the environmental degradation product of Ziram as well as the ferric dimethyldithiocarbamate, Ferbam. The genotoxicity profile for Thiram is similar to Ziram (, positive for gene mutations in *S. typhimurium* TA 1535 and TA100, clastogenic in CHEL and CHO cells at noncytotoxic S9-activated doses but with a preponderance of unstable structural chromosomes, no alkylating activity toward select nucleophiles) and causes increased thyroid gland C-cell hyperplasia in male rats (MRID No. 4215601). In contrast to Ziram, however, Thiram was shown to induce reproducible increases in micronuclei in the bone marrow cells of male mice. There is also evidence from the open literature and the submitted studies that Thiram causes decreased fertility in rats, embryotoxicity in rats and hamsters and teratogenicity in mice and hamsters. Other structural analogues (e.g., ferbam and the sodium and potassium salts of dimethyldithiocarbamate) were also positive in *S. typhimurium* TA 1535 and TA100; negative for SCE induction in mammalian cells, and either negative or yielded equivocal results for mammalian cell gene mutations in CHO cells. Both the sodium and potassium salts produced conflicting results in the developmental toxicology studies that were performed (i.e, Na was negative in both rabbits and rats while K was negative in the rat but induced malformations and other adverse fetal effects in the rabbit). No data were available on the carcinogenic potential of either salt. Ferbam was also mutagenic in *S. typhimurium* TA1535 and TA 100. Short et al., (1976) found that ferbam has little or no adverse effect on reproduction and was judged to be not teratogenic in mice or rats. The International Agency for Research on Cancer (IARC, 1976) indicated that ferbam did not induce a carcinogenic effect in mice or rats but listed ferbam as Group 3 (i.e., unclassifiable as to carcinogenicity in humans).

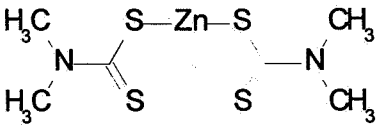
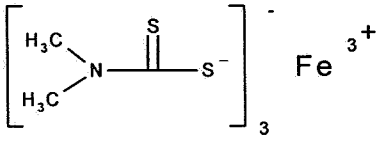
Despite the wealth of data on ziram and other dimethyldithiocarbamates, the only consistent finding among the studied members of this chemical class was a direct mutagenic effect on the base-pair substitution strains of *S. typhimurium*. In agreement

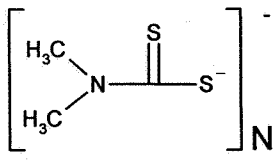
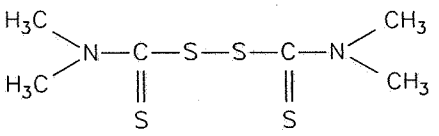
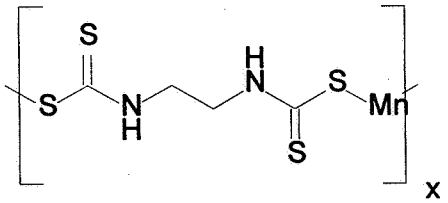
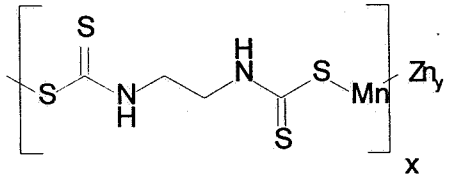
with the latter statement, Moriya et al. (1983) in their evaluation of pesticides in microbial systems found that 7 of the 13 dimethyl-dithiocarbamates studied did not require exogenous metabolic activation to induce mutations in *S. typhimurium* TA1535 or TA100 and that all of the positive dimethyldithiocarbamates share a common moiety, (CH3)2NCSS-, which appears to be essential for mutagenicity. While the dimethyl moiety may confer genotoxic activity, its role in the process of carcinogenicity and the potential electrophilicity of Ziram is not clear since lead dimethyldithiocarbamate and possibly ferbam are not carcinogenic but are positive in *S. typhimurium* TA100 (Zeiger 1987). Additionally, Ashby and Tennant (1991) list Ziram as a "non-alerting" carcinogen affecting a single species, sex and site. A similar conclusion can be reached for Thiram.

Since the structure of Ziram does not suggest electrophilicity and there was no evidence of acellular alkylation of nucleotides, attempts by several investigators to uncover the mechanism of mutagenic action toward bacteria have failed. Nevertheless, until a plausible explanation is obtained for the disparate results, the weight-of-the-evidence indicates that a mutagenic mode of action can not be ruled out for Ziram.

Table 5 summarizes the carcinogencity and mutagenicity of compounds structurally related to ziram and ferbam.

Table 5. Carcinogenicity of ziram / ferbam and structurally related compounds.

Compound	Structure	Carcinogenic Effect	Carcinogen Class/ Mutagen
Ziram		Hemangiomas in lymph nodes and spleen in male rats. NTP studies showed thyroid gland C-cell adenomas in male rats and lung tumors in female mice..	Not classified IARC - Group 3 Positive: Ames TA 1535, TA 100 Mouse lymphoma Clastogenic in CHO, CHEL, Human lympho.
Ferbam		No studies submitted to the Agency. NTP studies did not show any evidence of carcinogenicity.	Not classified IARC - Group 3 Positive: Ames TA 1535, TA 100

Compound	Structure	Carcinogenic Effect	Carcinogen Class/ Mutagen
Na, K, Pb salts	 Na^+ K^+ Pb^{2+}	No studies available.	Not classified Positive: Ames TA 100 All 3 salts
Thiram		No significant increase in tumor incidence in mice or rats.	Not classified Positive: Ames TA 1535, TA 100 Micronucleus Clastogenic in CHEL and CHO cells
Maneb (Mn) Nabam (Na) Zineb (Zn)		Maneb - Thyroid follicular cell adenomas and carcinomas in male and female rats. Nabam and Zineb - No information, but metabolite ETU is classified as B2.	Maneb - B2 Negative for mutagenicity except positive for SCE w/activation. Nabam - Not classified Negative: Ames Cell transformation *in vitro/in vivo* cytogenetic Positive: UDS, CHO/HGPRT, SCE Zineb - Not classified IARC - Group 3 Negative for mutagenic.
Mancozeb		Thyroid follicular cell adenomas and carcinomas in male and female rats.	B2 Negative for mutagenicity except positive SCE +/- UDS

4. Subchronic and Chronic Toxicity

A) Subchronic Toxicity

Rat

In a subchronic oral toxicity study (MRID 42450301), treatment-related increases in brain and spleen weights relative to body weight were seen in both sexes at 300 ppm and 1000 ppm, but with no concomitant histopathology. There was a slight increase in the incidence of centrilobular hepatocyte necrosis in one lobe in females at 300 ppm (1/10) and 1000 ppm (1/10) as compared to the controls (0/10). Also, increases in the incidence of localized epithelial hyperplasia in the stomach was noted in both sexes at 1000 ppm (1/10 males, 3/10 females) and in females at 300 ppm (1/10). Body weight gain decreased in both sexes at 300 ppm (82% of controls for both) and at 1000 ppm (67% for males, 68% for females). The LOAEL was 300 ppm (21.4/24.2 mg/kg/day in male/female) based on decreased body weight, body weight gain, and food consumption, and minimal histopathological changes in the liver (females). The NOAEL was 100 ppm (7.4/8.8 mg/kg/day in male/female). This study was classified as acceptable/guideline.

B) Chronic Toxicity

Rat

Refer to Rat Combined Chronic Toxicity/Carcinogenicity Study on page 1 of this report.

The LOAEL was 60 ppm (2.5/3.4 mg/kg/day in male/female) based on increased hemosiderosis in the spleen, increased epithelial hyperplasia and subepithelial edema in the non-glandular region of the stomach, increased incidence of narrowed peripheral muscle fiber bundles in skeletal muscle, and increased cortical hypertrophy with vacuolation in the adrenals in males and an increased incidence of prominent ultimobranchial cysts in the thyroid in females. No NOAEL was determined in this study.

Mouse

Refer to Mouse Carcinogenicity Study on page 6 of this report.

The LOAEL was 225 ppm (27/33 mg/kg/day for males/females) based on decreased absolute brain weights in both sexes and significantly increased incidence of urinary bladder epithelial hyperplasia and decreased body weight gain in males. The NOAEL was 75 ppm (9/11 mg/kg/day in male/femals).

Dog

In a chronic feeding study (MRID No. 42823901), Ziram (98.5%; Lot No. 8331 AA) was administered for 52 weeks in the diet to four male and four female beagle dogs per dose at concentrations of 0, 50, 185, and 700 ppm (700 ppm dose reduced to 500 ppm at day 3 of week 12), equivalent to doses of 0, 1.6, 6.6, 17.4 mg/kg/day for males and 1.9, 6.7, and 20.6 mg/kg/day for females, respectively. [Attachment 8]

There was a treatment-related convulsive episode at week 11 for a female in the 700/500 ppm dose group that required the animal to be euthanitized. In addition to the convulsive episode, the findings for the 700/500 ppm and 185 ppm dose groups include: 1) decreased body weight gain for females over the treatment period, 2) changes in clinical chemistry parameters-decreases in albumin (males and females) and total protein levels (females) and increases in SGPT (males) and alkaline phosphatase (males), and 3) histologic findings for livers (aggregates of Kupffer cells and macrophages and increased infiltration of inflammatory cells in males and females) and spleens (pigmented macrophages; males). The NOAEL is 50 ppm based on the lack of significant toxicological effects. The LOAEL is 185 ppm based on decreased body weight gain in females, and increased liver pathology accompanied by corresponding clinical chemistry changes and the occurrence of pigmented macrophages in the spleen in males.

5. Mode of Action Studies

No mode of action studies were submitted.

V. WEIGHT-OF-THE-EVIDENCE CONSIDERATIONS

VI. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

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ATTACHMENTS

Attachment 1	Data waiver request for ferbam
Attachment 2	Combined Chronic/Carcinogenicity Feeding Study - Rat
Attachment 3	Historical Control Data - Incidence of lymph node and spleen hemangiomas in male rats
Attachment 4	Qualitative Risk Assessment on Combined Chronic/Carcinogenicity Rat Feeding Study
Attachment 5	Carcinogenicity Feeding Study - Mouse
Attachment 6	Abstract on NTP carcinogenesis bioassay of ziram and table of primary tumors in male rats
Attachment 7	Table of primary tumors in female mice
Attachment 8	Chronic Oral Toxicity Feeding Study - Dog

Attachment 1

JAN 2 4 1991

HED Project #: 0-82180 Date In: 1/2/91

Due Date: 1/17/91 Caswell #: 458

Branch: IRSB # of Beans: 1

Chemical Manager: _____ Farbam - ID # 034801-045728 Fifra 88

Please return to SACB (M. Hawkins) by Friday.

HED Project #: 0-82180

Branch: IRSB

Section Head: _____

Reviewer: P. Errico

Due Date: ~~1/17/91~~ 2/11/91

Can you meet or better this date? Yes? _____ No? _____

If not what date can you meet? _____

Studies: 12 Fifra 88

Total Estimated Completion Hours (TECH): 8

HED.1-8/12/88

CASE: 803617
SUBMISSION: S388171

DATA PACKAGE RECORD
BEAN SHEET

DATE: 12/31/9
Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REREGISTRATION ACTION: 603 PHASE 3 INITIAL SUB
CHEMICAL: 034801 Ferbam (ferric dimethyldithiocarbamate)
ID#: 034801-045728
COMPANY: 045728 U C B CHEMICALS CORP.
PRODUCT MANAGER: 50 [redacted] 703-308-8085 ROOM: CST 4J1
PM TEAM REVIEWER: TOM MYERS 703-308-8074 ROOM: CST 4N1
RECEIVED DATE: 12/21/90 DUE OUT DATE: / /

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 159794 EXPEDITE: N DATE SENT: 12/27/90 DATE RET.: / /
DP TYPE: 101 Phase IV Review
ADMIN DUE DATE: 01/17/91 CSF: N LABEL: N

ASSIGNED TO	DATE IN	ASSIGNED TO	DATE IN
DIV : HED	1/2/91	REVR :	/ /
BRAN: TB-IRS	/ /	CONTR:	/ /
SECT:	/ /		

* * * DATA PACKAGE REVIEW INSTRUCTIONS * * *

CASE 2180. Beginning MRID# 92038000.

8 of 8 submissions for this case.

For the attached reregistration case, please identify all applicable data requirements and note those for which adaquate data have not been submitted to the Agency.

Request for data waivers and request to use analog data attached for review.

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL
159790	EEB	12/27/90	01/17/91	Y	N	N
159791	EFGB	12/27/90	01/17/91	Y	N	N
159792	DEB	12/27/90	01/17/91	Y	N	N
159793	NDEB	12/27/90	01/17/91	Y	N	N

PHASE FOUR REVIEW

(NOTE: This only contains additions and changes from the phase 2 response.)

Pesticide: Ferbam

Transmitted to HED on: 1/2/91 Chemical#/Case#: 2180/803617
Tox. Chem #: 458 Sponsor: UCB Chem. Corp.

CRM: Tom Myers Phone#: 703-308-8074

Branch: Toxicology Branch I Reviewer: Pam Hurley

Completed: / / Concurrence: Roger Gardner 2/8/91

Are there any changes from the reviews in phase 2? ____ NO X YES (See below)

Response, by Guideline

Guideline #: 81-1 Acute oral/rat

MRID 40561501/92038008 **Study #**NOTOX 0740/930
Discussion/Recommendation:

Summary acceptable by acceptance criteria. Study itself needs to be reviewed.

Guideline #: 81-2 Acute dermal/rabbit

MRID 40561502/92038010 **Study #**NOTOX0740/931
Discussion/Recommendation:

Summary acceptable by acceptance criteria. Study itself needs to be reviewed.

Guideline #: 81-3 Acute inhalation/rat

MRID 41508101/92038011 **Study #** UCB 285/88179
Discussion/Recommendation:

Summary acceptable by acceptance criteria. Study itself needs to be reviewed.

Guideline #: 81-4 Primary eye irritation/rabbit

MRID 40561503/92038013 **Study #** NOTOX 0940/933
Discussion/Recommendation:

Summary acceptable by acceptance criteria. Study itself needs to be reviewed.

Guideline #: 81-5 Primary dermal irritation/rabbit

MRID 40561505/92038027 **Study #** NOTOX 0740/932
Discussion/Recommendation:

Summary acceptable by acceptance criteria. Study itself needs to be reviewed.

Guideline #: 81-6 Dermal sensitization/Guinea Pig

MRID 40561504/92038029 **Study #** NOTOX 0740/934
Discussion/Recommendation:

Summary acceptable by acceptance criteria. Study itself needs to be reviewed.

Guideline #: 82-1a 90-day feeding/rodent
MRID_____ **Study #**_____
Discussion/Recommendation:

Previously committed to supply this study for ziram. Request for using this study to satisfy testing requirements for ferbam accepted. Justification adequate.

Guideline #: 82-1b 90-day feeding/nonrodent

MRID_____ **Study #**_____
Discussion/Recommendation:

Previously committed to supply this study for ziram. Request for using this study to satisfy testing requirements for ferbam accepted. Justification adequate.

Guideline #: 82-2 — 21 Day dermal/rodent/rabbit

MRID_____ **Study #**_____

Discussion/Recommendation:

Registrant requested waiver on the basis of low acute dermal toxicity, worker protection requirements and due to the fact that the formulation is a dust-free granule thus reducing exposure during product use. Waiver is accepted, not because of reasons given by Registrant, but because a 21-day dermal study is available for ziram, which can be used for ferbam.

Guideline #: 83-1a — Chronic toxicity/rodent

MRID_____ **Study #**_____

Discussion/Recommendation:

Same comments apply as for 90-day feeding/rodent.

Guideline #: 83-1b — Chronic toxicity/nonrodent

MRID_____ **Study #**_____

Discussion/Recommendation:

Same comments apply as for 90-day feeding/rodent.

Guideline #: 83-2a — Oncogenicity/rat

MRID_____ **Study #**_____

Discussion/Recommendation:

Same comments apply as for 90-day feeding/rodent.

Guideline #: 83-2b — Oncogenicity/mouse

MRID_____ **Study #**_____

Discussion/Recommendation:

Same comments apply as for 90-day feeding/rodent.

Guideline #: 83-3a — Teratology/rat

MRID_____ **Study #**_____

Discussion/Recommendation:

Same comments apply as for 90-day feeding/rodent.

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Guideline #: 83-3b — Teratology/rabbit

MRID_____ **Study #**_____
Discussion/Recommendation:

Same comments apply as for 90-day feeding/rodent. Study is available on ziram and has been accepted for review using acceptance criteria.

Guideline #: 83-4 — Two-generation reproduction/rat

MRID_____ **Study #**_____
Discussion/Recommendation:

Same comments apply as for 90-day feeding/rodent. Ziram study not acceptable new study required.

Guideline #: 84-2a — Mutagenicity/Ames

MRID_____ **Study #**_____
Discussion/Recommendation:

Same comments apply as for 90-day feeding/rodent.

Guideline #: 84-2b — Mutagenicity/Struct. Chromosomal Aberration

MRID_____ **Study #**_____
Discussion/Recommendation:

Same comments apply as for 90-day feeding/rodent.

Guideline #: 84-4 — Other genotoxic effects

MRID_____ **Study #**_____
Discussion/Recommendation:

Same comments apply as for 90-day feeding/rodent.

Guideline #: 85-1 — Metabolism

MRID_____ **Study #**_____
Discussion/Recommendation:

Same comments apply as for 90-day feeding/rodent. Some data already available on ferbam which will be used in conjunction with data on ziram.

Guideline #: 85-2 Dermal penetration

MRID_____ **Study #**_____

Discussion/Recommendation:

Not required unless we have a toxicity end-point which indicates that we need this study.

Attachment 2

DATA EVALUATION REPORT

ZIRAM

Study Type: CHRONIC FEEDING/ONCOGENICITY- RAT (83-5)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Health Sciences Research Division
Oak Ridge National Laboratory*
Oak Ridge, TN 37831
Task Order No. 94-430 M

Primary Reviewer: C. S. Jamison, Ph.D.	Signature: RHR for C.S. Jamison Date: 7-14-95
Secondary Reviewers: C. B. Bast, Ph.D., D.A.B.T.	Signature: CB Bast Date: 7-14-95
K.A. Davidson, Ph.D., D.A.B.T.	Signature: H.T. Boyce for K.A. Davidson Date: 7/18/95
Robert H. Ross, M.S., Group Leader	Signature: RHR Date: 7-14-95
Quality Assurance: Susan Chang, M.S.	Signature: SMS Chang Date: 7/14/95

Disclaimer

This final Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Oak Ridge National Laboratory personnel.

*Managed by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under Contract No. DE-AC05-84OR21400

There was no excess mortality in any of the treated groups relative to controls. Group mean body weight gains were decreased for males (86% of control, $p<0.01$ and females (74% of control, $p<0.01$) in the high dose group (540 ppm). Food consumption was decreased compared to controls for males (540 ppm: 91%, $p<0.01$) and females (180 ppm: 92%, $p<0.05$; 540 ppm: 94%, $p<0.05$). Hematology parameters (RBC, HGB, and PCV) were decreased relative to controls for females in the 540 ppm (weeks 26-104, $p<0.05$, $p<0.01$) and 180 ppm (weeks 26-52, $p<0.05$, $p<0.01$)) dose groups. There were statistically significant decreases ($p<0.05$, $p<0.01$) in clinical chemistry parameters (calcium, total protein, albumin, calcium and SGPT) during weeks 13-52 for females. For males (540 ppm, week 104) organ weight for the adrenals was decreased (absolute, 59% of control, $p<0.01$; relative, 67% of control, $p<0.05$). There were macroscopic pathological findings (not statistically significant) for animals in the 180 and 540 ppm dose groups for the stomach and skeletal muscle (males and females), and the adrenals (females only). There were microscopic pathological findings for males and females in the 180 and 540 ppm dose groups for spleen ($p<0.01$), liver ($p<0.01$, $p<0.05$), stomach ($p<0.05$, $p<0.01$), thyroid ($p<0.01$, $p<0.05$), skeletal muscle ($p<0.01$), spinal cord (males only, $p<0.05$), sciatic nerve (females only, $p<0.01$), and adrenal cortex ($p<0.05$, $p<0.01$). As there were histopathological findings for males in the 60 ppm dose group for spleen ($p<0.01$), stomach ($p<0.01$, $p<0.05$), skeletal muscle ($p<0.05$), and adrenal cortex ($p<0.05$), a NOAEL for males could not be identified. For females, there was an increase in prominent ultimobranchial cysts in the thyroid in all dose groups (Controls: 3/50; 60 ppm: 12/50, $p<0.05$; 180 ppm: 22/50, $p<0.01$;, 540 ppm: 27/50, $p<0.01$), precluding the identification of a NOAEL for females. **The NOAEL could not be identified for either males or females, due to histopathological findings for animals in the low dose group (60 ppm).**

Carcinogenic potential was evidenced by the finding of treatment-related tumors (benign hemangioma) in mesenteric lymph nodes (5/50, $p<0.05$) and in spleen (1/50) in males in the 540 ppm dose group. There were no treatment-related tumors identified in males in the 180 or 60 ppm dose groups, or in females in any dose group. There were no treatment-related malignant tumors in either sex. The dosing is adequate. Treatment of males with Ziram for 104 weeks at the MTD resulted in neoplastic changes.

This study is classified as Acceptable and satisfies the guideline requirements for a chronic/oncogenicity study (§83-5). This study did not establish a NOAEL.

Special Review Criteria (40 CFR 154.7) None

hematologic and clinical chemistry changes for the low dose group (100 ppm). There were effects on weight gain and food intake and minor hematological, clinical chemistry, organ weight and pathological changes for the medium and high dose groups (300 and 1000 ppm, respectively). The low dose for the current study was chosen to be 60 ppm, in an attempt to provide a NOEL. The medium and high doses were set at 3 and 9 times this dose (180 and 540 ppm, respectively).

TABLE 1. STUDY DESIGN

Dose Group	Doses (mg/kg/day)			No. Animals	
	Target Dose Both Sexes	Dosage Achieved (Mean[a])		Male	Female
		Males	Females		
1 Control[b]	0 ppm	0	0	50	50
2 Low (LDT)[b]	60 ppm[c]	2.5	3.4	50	50
3 Mid (MDT)[b]	180 ppm	7.7	10.2	50	50
4 High (HDT)[b]	540 ppm	23.7	34.6	50	50
5 Satellite Control[d]	0 ppm	0	0	20	20
6 Satellite (LDT)[d]	60 ppm[c]	3.0	3.9	20	20
7 Satellite (MDT)[d]	180 ppm	9.1	11.7	20	20
8 Satellite (HDT)[d]	540 ppm	27.3	37.5	20	20

Data obtained from summary table on p. 43, MRID No. 43404201.
[a]Compound intake was calculated by the study authors on a weekly basis. The mean of the weekly values over the 104 week test period is presented.
[b]Data for weeks 1-104: includes combined data for main and satellite groups during weeks 1-52 and data for main group during weeks 53-104.
[c]Diet prepared contained 66 ppm.
[d]Achieved dose for satellite groups alone was not presented in the study report (MRID No. 434042-01). Data was combined by the study authors for satellite and main groups for weeks 1-52

2. Diet preparation and analysis

Diet was prepared weekly. A concentrate was prepared by grinding appropriate amounts of Ziram with untreated sieved basal diet and mixing in a Turbula mixer for at least 2 minutes. The concentrated diet was diluted with appropriate quantities of untreated diet and homogeneity was achieved by mixing in a double-cone blender for at least 7 minutes. The total volume of diet required was large, such that the diets for each dose were prepared in 2 batches. Trial studies evaluating the stability of Ziram in rodent diet formulations showed that the compound was not stable at low doses (70 ppm) when stored at ambient temperature,

following sequence. Invalid values (documented by machine fault, organ loss or damage) are excluded. If the relative frequency of the mode is greater than 75%, the proportion of animals with values different from the mode were analyzed. Otherwise, Bartlett's test was used for analysis of heterogeneity of variance between treatments. If there was significant (1% level) heterogeneity, the data were transformed logarithmically. If no significant heterogeneity was found, or if the data transformation was satisfactory, a one-way analysis of variance was performed. If significant heterogeneity of variance was present and could not be removed by logarithmic transformation, the Kruskal-Wallis' analysis of ranks was used. Analyses of variance were followed by Student's *t*-test and Williams' test for a dose-related response. Kruskal-Wallis' test was followed by non-parametric equivalents of the *t*-test and Williams' test (Shirley's test). Where appropriate, an analysis of covariance was used in place of an analysis of variance. For organ weight data, an analysis of variance was performed using terminal body weight as a covariate when the within-group relationship between organ weight and body weight was significant at the 10% level. Mortality was analyzed using log rank methods. Incidence of tumors was analyzed according to the context of the observation as interpreted by the pathologist. Trend tests were used based upon nominal dose levels.

5. Signed and dated GLP/quality assurance statements were present.

C. METHODS AND RESULTS

1. Observations

Animals were palpated and inspected daily during the first 4 weeks and once a week thereafter for signs of behavioral changes, reactions to treatment, and ill health. Checks for dead and/or moribund animals were performed twice daily.

Results – There were no clinical signs indicative of a response to treatment. The mortality distribution did not indicate an adverse effect of Ziram treatment (Table 2). Mortality for females in the 180 (p=0.035) and 540 (p=0.029) ppm dose groups was statistically significantly decreased relative to controls. This was likely due to decreased survival for the control group (32%, expected 50% at 104 weeks). The overall test for trend was not significant.

TABLE 3. GROUP MEAN BODY WEIGHTS (G/RAT) AT SELECTED WEEKS & GROUP MEAN BODY WEIGHT CHANGES (G/RAT) AT SELECTED WEEKLY INTERVALS

Week of Study	Males				Females			
	0	60[a]	180	540	0	60[a]	180	540
0	174	173	174	174	142	142	142	144
1	222	219	208	186	168	168	163	150
4	332	331	318	285	220	220	214	193
13	485	490	477	428	282	283	275	252
26	597	607	584	522	332	333	311	287
52	707	730	694	620	426	424	383	338
104	752	740	697	671	470	466	465	388
Weeks 0-1	48.4	45.9 (95%[b])	34.1** (70%)	12.2** (25%)	25.6	26.0 (102%)	20.6** (80%)	5.8** (23%)
Weeks 0-52	534	557 (104%)	520 (97%)	446** (84%)	284	282 (99%)	241** (85%)	194** (68%)
Weeks 1-104	528	520 (98%)	489 (93%)	486 (92%)	304	303 (100%)	302 (99%)	238* (78%)
Weeks 0-104	576	565 (98%)	523 (91%)	496** (86%)	329	326 (99%)	323 (98%)	245** (74%)

Data adapted from summary tables, pp. 39-40 and Table 2, pp. 72-75, Appendix 2, pp. 303-446, MRID No. 42450301.
[a]Diet prepared to contain 66 ppm.
[b]% of control
*$p<0.05$, **$p<0.01$, Data for weeks 0-52 included both main and satellite group animals. For statistical analysis data for females for weeks 0-52 were log-transformed. For weeks 0-1, 1-104, and 0-104 (males), Kruskal-Wallis analysis of mean ranks was applied.

3. Food consumption and compound intake

Food consumption for each cage was determined daily and reported as food intake (g) per rat per week, based upon the number of surviving animals in the cage. Mean daily diet consumption was calculated by the reviewer as g food/kg body weight/day. Food efficiency (body weight gain, kg/food consumption, kg per unit time X 100) was calculated by the reviewer. Compound intake (mg/kg/day) values were calculated as time-weighted averages from the food consumption and mid-week body weight data.

Results –

a. Food consumption – Food consumption (Table 4) was statistically signficantly decreased for males in the 540 ppm dose group (91% of control, $p<0.01$) and females in the 180 (92% of control, $p<0.05$) and 540 ppm dose groups (94% of control, $p<0.05$). Food consumption for rats treated with Ziram at 60 ppm was similar to controls.

5. Blood was collected during weeks 13, 26, 52, 78, and at termination under ether anesthesia and after overnight fast from the orbital sinus of 10 male and 10 female rats from each dose group for hematology and clinical analysis. Rats from the satellite group were used for samples obtained in weeks 13, 26, and 52. Rats from the main group were used for the week 78 and termination samples. Where possible, the same rats were used for drawing blood. In week 4, blood samples were withdrawn from the orbital sinus of 10 male and 10 female rats for T3, T4, and TSH measurements. Blood samples were collected and mixed with EDTA for hematology, citrate for blood coagulation tests, or heparin for clinical chemistry evaluations. The CHECKED (X) parameters were examined.

a. Hematology

X			X		
X	Hematocrit (HCT)*		X	Leukocyte differential count*	
X	Hemoglobin (HGB)*		X	Mean corpusc. HGB conc. (MCHC)	
X	Leukocyte count (WBC)*		X	Mean corpusc. volume (MCV)	
X	Erythrocyte count (RBC)*			Reticulocyte count	
X	Platelet count*				
X	Blood clotting measurements				
	(Thromboplastin time)				
X	(Clotting time)				
	(Prothrombin time)				

* Required for subchronic and chronic studies.

Results – There were changes in the hematological parameters RBC, HCT, HGB, MCHC, MCV, and clotting time that were statistically significantly different from control values (Table 5, $p<0.05$ and $p<0.01$). The most consistent change was decreased RBC levels relative to controls. The decreases in RBC were evident for males (180, 540 ppm) at week 13 and for females treated with Ziram at 180 ppm at weeks 26 and 52 and at 540 ppm from week 26 to 104. HGB and PCV values were correspondingly decreased at most of these timepoints and the decreases were generally statistically significant ($p<0.05$, $p<0.01$). The values for all of the hematological parameters fell within the historical control range established by the testing facility. The only statistically significant change for animals treated with Ziram at 60 ppm was a decreased blood clotting time for males at week 26 ($p<0.05$).

b. Clinical chemistry

X	Calcium*
X	Chloride*
	Magnesium
X	Phosphorus*
X	Potassium*
X	Sodium*

Enzymes

X	Alkaline phosphatase (ALK)
	Cholinesterase (ChE)
	Creatinine phosphokinase
	Lactic acid dehydrogenase (LDH)
X	Serum alanine aminotransferase (also SGPT)*
X	Serum aspartate aminotransferase (also SGOT)*
X	Glutamic-oxaloacetic transaminase (GOT)
	Glutamate dehydrogenase

X	Albumin*
X	Blood creatinine*
X	Blood urea nitrogen*
X	Cholesterol*
X	Globulins
X	Glucose*
X	Total bilirubin
X	Total serum protein (TP)*
	Triglycerides
	Serum protein electrophoresis
X	Tri-iodothyronine (T3)
X	Thyroxine (T4)
X	Thyroid Stimulating Hormone (TSH)

* Required for subchronic and chronic studies.

Results – For males (Table 6), there were statistically significant decreases relative to controls in total serum protein (94-98.5% of control, $p \leq 0.05$), albumin (93-96.7% of control, $p \leq 0.05$, $p \leq 0.01$), SGPT (66.7-77% of control, $p \leq 0.05$, $p \leq 0.01$), calcium (96-98% of control, $p \leq 0.05$, $p \leq 0.01$), and T4 (75.7-85% of control, $p \leq 0.05$, $p \leq 0.01$) levels and increases relative to controls in urea (115-121% of control, $p \leq 0.05$) and ALK (125-142% of control, $p \leq 0.05$) levels. For females, there were statistically significant decreases relative to controls in total serum protein (93-95.7% of control, $p \leq 0.05$, $p \leq 0.01$), albumin (91-88.6% of control, $p \leq 0.05$, $p \leq 0.01$), SGPT (43-44% of control, $p \leq 0.05$, $p \leq 0.01$), SGOT (26% of control, $p \leq 0.05$), calcium (94-96% of control, $p \leq 0.05$, $p \leq 0.01$), and T4 (74% of control, $p \leq 0.05$) levels and increases in urea (142-143% of control, $p \leq 0.01$), chloride (101-102% of control, $p \leq 0.05$, $p \leq 0.01$) levels. The increases in ALK levels were dose-dependent for both males and females. There was a dose-dependent decrease in SGOT levels for females at weeks 26 and 52. SGOT levels for females in the control and 60 ppm dose groups were higher than males in these dose groups at weeks 26 and 52. Thus, SGOT levels for females in the 540 ppm dose group at week 26 were statistically significantly (26% of control, $p \leq 0.05$) lower than controls. The only statistically significant changes affecting rats in the 60 ppm dose group were a decrease in total protein levels for males at week 52, relative to control (97.1% of control, $p \leq 0.05$), and an increase in chloride levels for females at week 13, relative to control (101% of control, $p \leq 0.05$).

TABLE 6 (Continued)

Parameter	Week of Study	Exposure Level (ppm)							
		Males (N=10)				Females (N=10)			
		0	60[a]	180	540	0	60[a]	180	540
SGOT	13	54	54	51	52	82	62	57	53
	26	50	55	51	50	207	102	77	53*
	52	56	55	56	53	159	84	75	71
	78	53	61	59	45	77	73	90	63
	104	51	55	75	50	77	78	97	80
Calcium	13	5.4	5.5	5.4	5.2**	5.4	5.4	5.3*	5.1**
	26	5.5	5.5	5.5	5.4*	5.6	5.6	5.5	5.4**
	52	5.6	5.6	5.5	5.5	5.6	5.6	5.7	5.4**
	78	5.5	5.4	5.4**	5.4**	5.4	5.6	5.4	5.4
	104	5.4	5.4	5.3	5.3	5.4	5.6	5.3	5.3
Chloride	13	101	101	102	100	101	102*	103*	102*
	26	101	101	100	100	100	101	102**	102**
	52	100	102	101	101	99	100	100	101*
	78	102	103	102	102	99	100	100	101*
	104	100	100	100	101	98	96	97	98
T4	4	3.7	3.4	3.0**	2.8**	2.7	2.4	2.4	2.0*
	13	3.7	3.4	3.2	3.2	2.9	2.7	2.7	2.6
	26	3.4	3.3	3.1	2.9*	2.3	2.4	2.5	2.4

Data taken from summary table, p. 47, MRID No. 42434001. Parameter unit are: Total serum protein (g/dL), Albumin (g/dL), Urea (blood urea nitrogen, mg/dL), ALK (mU/mL), SGPT (mU/mL), SGOT (mU/mL), Ca^{+} (mEq/L), Cl^{-} (mEq/L), T4 (μg/dL).
[a]Diet prepared to contain 66 ppm.
*$p \leq 0.05$
**$p \leq 0.01$

6. Urinalysis

Urine was collected in an unspecified manner from 10 male and 10 female animals per dose group during weeks 13, 26, 52 (satellite group), 78, and at termination (main group). Food and water were removed during the overnight collection period (~16 hours). The CHECKED (X) parameters were examined.

TABLE 7. SUMMARY OF STATISTICALLY SIGNIFICANT CHANGES IN URINALYSIS PARAMETERS IN RATS ADMINISTERED ZIRAM FOR 104 WEEKS.

Parameter	Week of Study	Treatment Group/Exposure Level (ppm)							
		Males (N=10)				Females (N=10)			
		0	60[a]	180	540	0	60[a]	180	540
*p*H	13	7.0	6.9	7.3	7.5**	6.4	6.4	6.5	6.5
	26	6.9	7.1	7.6**	7.7*	6.5	6.6	6.5	6.6
	52	6.8	6.9	7.3*	7.3*	6.2	6.4	6.4	6.3
	78	6.9	7.1	7.0	7.4*	6.2	6.4	6.3	6.6*
	104	6.4	6.6	6.2	6.7	6.3	6.2	6.4	6.4
Volume	13	6.2	6.1	6.0	5.6	2.6	3.1	2.7	2.0
	26	5.1	6.7	6.9	6.9	3.2	3.1	2.5	3.2
	52	6.3	7.1	5.9	5.9	6.6	5.1	5.6	3.5*
	78	10.6	10.4	9.0	7.6	6.6	8.2	8.4	6.9
	104	9.4	7.1	10.4	8.8	10.7	8.3	12.9	10.6
Protein	13	175	171	188	168	81	90	82	75
	26	201	177	135	132	73	83	70	66
	52	272	220	215	334	81	142	134	87
	78	336	116	188	114	518	107	100	100
	104	552	289	929	477	337	652	200	73*

Data taken from Table 9, pp. 107-111, MRID No. 42434001. Parameter units are: Volume (mL), Protein (mg/dL).
[a]Diet prepared to contain 66 ppm.
*$p \leq 0.05$
**$p \leq 0.01$

Results –

a. Organ weight – For males (Table 8), absolute organ weight for adrenals were dose-dependently decreased at weeks 52 and 104. The decrease was statistically significant at week 104 for males in the high dose group for absolute (58.8% of control, $p<0.01$) and relative (66.7% of control, $p<0.05$) adrenal weights. Relative brain weights for males in the 540 ppm dose group were statistically significantly increased at week 104 (110% of control, $p<0.05$). Relative testes weights were statistically significantly increased at week 52 for males in the 540 ppm dose group (115% of control, $p<0.05$) and at week 104 for males in the 180 (112.5% of control, $p<0.05$) and 540 ppm (127% of control, $p<0.01$) dose groups. Relative organ weights were statistically significantly increased for females in the high dose group relative to controls for brain (weeks 52 and 104, 120% of control, $p<0.01$), thyroid (week 104, 133% of control, $p<0.05$), heart (week 52, 117% of control, $p<0.01$; week 104, 112% of control, $p<0.05$), kidney (week 52, 111% of control, $p<0.05$), and adrenal (week 104, 156% of control, $p<0.01$). For females, relative liver weights were dose-dependently increased at weeks 52 and 104. The increases were statistically significant at week 52 for females in the 60, 180, and 540 ppm dose groups (113%, 113%, and 120% of control, respectively, $p<0.01$) and at week 104 for females in the 540 ppm dose group (112% of control, $p<0.05$). Relative pituitary weights were statistically significantly decreased (72.5% of control, $p<0.05$) at week 52 for females in the 540 ppm dose group.

b. Gross pathology – Treatment related findings (Table 9) occurring at a higher incidence in treated animals than in controls included depressions, raised areas, thickening, and white discoloration in the forestomach, cysts and cystic enlargement in adrenals (females only), and atrophy of hindlimbs. The incidence of these findings were not statistically significantly different from controls, however, there are correlates with microscopic pathological findings for stomach, adrenals, skeletal muscle, spinal cord, and sciatic nerve. There was also an increased incidence of absence of corpora lutea for females treated with Ziram at 60, 180, and 540 ppm (44%, 48%, 62%, respectively) as compared to controls (38%). This finding is not likely toxicologically significant. In the microscopic pathological examination of the ovaries, there was a similar incidence of absence of corpora lutea in treated animals as compared to controls (see Table 10 of this report).

TABLE 9. MACROSCOPIC PATHOLOGY INCIDENCE SUMMARY FOR RATS ADMINISTERED ZIRAM FOR 104 WEEKS.

Pathology	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	60	180	540	0	60	180	540
No. animals	50	50	50	50	50	50	50	50
Ovaries								
-no corpora lutea visible	---	---	---	---	19	22	24	31
Forestomach								
-Depression	11	16	20	20	8	7	14	31
-Raised Areas	0	0	3	4	0	1	1	0
-Thickening	5	11	11	12	3	3	8	12
-White Discoloration	4	8	7	6	1	1	4	9
Overall Pathology Incidence for Forestomach	20	35	41	42	12	12	27	52
Adrenals								
-Cysts/Cystic	0	0	0	0	0	0	1	5
-Cystic enlargement	0	0	0	0	0	0	0	2
Skeletal muscle								
-Atrophied hindlimbs	4	5	3	6	0	0	0	2

Data adapted from summary table, p. 50, MRID No. 42434001.

TABLE 10. MICROSCOPIC PATHOLOGY: NON-NEOPLASTIC CHANGES FOR MAIN GROUP RATS ADMINISTERED ZIRAM FOR 104 WEEKS

Pathology	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	60	180	540	0	60	180	540
No. animals (main group)	50	50	50	50	50	50	50	50
Spleen								
No abnormalities detected	22	13*	13*	14	14	9	2**	3**
Hemosiderosis	10 (2.3)[a]	22** (1.6)	29** (2.0)	23** (2.0)	24 (2.8)	29 (2.2)	38** (2.3)	39** (2.9)
Liver								
Pigment (hemosiderin) in sinusoidal cells	2 (1.5)	5 (1.2)	22** (1.3)	26** (1.4)	0 (0)	3 (1.3)	13** (1.8)	15** (1.9)
Bile duct hyperplasia	7 (1.9)	7 (2.0)	13 (2.1)	15* (2.1)	5 (1.8)	6 (1.8)	9 (2.1)	17** (2.1)
Stomach-Non-Glandular Region								
No abnormalities detected	31	28	18**	20*	32	35	27	11**
Epithelial hyperplasia	6 (2.5)	18** (2.7)	25** (2.6)	25** (2.7)	7 (2.3)	6 (2.5)	15* (2.5)	37** (2.5)
Subepithelial edema	2 (3.0)	8* (2.75)	8* (2.75)	10* (2.7)	2 (2.5)	3 (3.3)	3 (2.3)	11** (2.7)
Ulceration	5 (2.4)	8 (2.4)	14* (2.4)	14* (2.4)	3 (2.3)	5 (2.8)	6 (2.5)	12* (2.2)
Perforating ulceration, marked	0	1	0	3	0	0	0	1
Hyperplasia at the limiting ridge	1 (2.0)	2 (2.0)	3 (2.0)	3 (2.3)	1 (2.0)	2 (2.5)	4 (2.8)	1 (2.0)
Pancreas								
Replacement by adipose tissue	7 (2.4)	14 (2.4)	15* (2.3)	21** (2.7)	0/50 (0)	2/50 (1.5)	4/49 (2.0)	3/50 (2.7)
Thyroid								
No abnormalities detected	29	31	25	24	37	31	20**	17**
C-cell hyperplasia	1 (2.0)	3 (2.3)	5 (2.2)	8* (2.5)	5 (2.8)	5 (2.2)	5 (2.2)	3 (2.3)
Prominent ultimobranchial cysts	4	5	14**	15**	3	12*	22**	27**
Parathyroids								
No abnormalities detected	48/49	40*/47	44/48	39*/46	47/48	46/46	46/47	47/47
Hyperplasia	1/49 (2.0)	4/47 (2.5)	4/48 (2.3)	7*/46 (2.3)	0	0	0	0

Appendix 2). The tumors with increased or decreased incidence relative to control are presented in Table 11. The incidence of males with tumors was decreased dose-dependently compared to controls (540 ppm, $p<0.05$). This decrease was primarily due to a dose-dependent decrease in malignant tumors for males (540 ppm, $p<0.01$). The only tumors considered by the study authors to be treatment-related were hemangiomas in mesenteric lymph nodes and spleens of males. These tumors were benign, showing no evidence of malignancy. The incidence of animals with this tumor type was statistically significantly increased for males treated with Ziram at 540 ppm ($p=0.024$), as compared to controls. In addition, the dose-related trend in the number of animals with this tumor type was statistically significant ($p=0.0001$). Historical control data obtained by the testing facility indicated that the incidence for this tumor type in males ranged from 0/50 to 2/50. The overall incidence for this tumor type in the current study was 6/50. For females, there were no tumors found with a higher incidence in treated animals than in controls that were considered by the study authors to be treatment-related. Appendix 2 summarizes tumor types and incidences in male and female rats of the main study groups.

TABLE 11. MICROSCOPIC PATHOLOGY: NEOPLASTIC CHANGES FOR MAIN GROUP RATS ADMINISTERED ZIRAM FOR 104 WEEKS

Neoplasia	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	60	180	540	0	60	180	540
No. animals	50	50	50	50	50	50	50	50
No. of animals with tumors[a]	45	44	41	37*	48	46	49	47
No. of animals with malignant tumors[a]	23	20	17	8**	12	15	21*	13
No. of animals with benign tumors[a]	35	34	32	35	46	41	42	43
No. of animals with metastatic tumors[a]	3	1	1	2	0	0	2	1
Lymph Nodes-Mesenteric								
Hemangioma (Benign)[b]	0	0	0	5*	0	0	0	0
Spleen								
Hemangioma (Benign)[b]	0	0	0	1	0	0	0	0

Data adapted from Table 13, p. 142-296, MRID No. 42434001.
[a]Statistical significance calculated by the reviewer, *t*-test.
[b]Statistical significance calculated by the study authors.
*$p<0.05$
**$p<0.01$

D. DISCUSSION

From the results presented in the current study report, MRID No. 434042-01, oral administration of Ziram at 60, 180, and 540 ppm for 104 weeks to male and female rats resulted in

bundles, $p<0.05$), and adrenal cortex (hypertrophy with vacuolation, $p<0.05$). These histopathologic changes were present in generally greater severity in the higher dose groups (180 and 540 ppm), were not present in controls, and thus are toxicologically significant. For females treated with Ziram at the low dose (60 ppm), the only treatment-related effect was a statistically significant increase ($p<0.05$) in the incidence of prominent ultimobranchial cysts in the thyroid. The toxicological significance of this finding is not clear. There were no statistically significant differences between controls and females treated with Ziram at 60 ppm in absolute or relative organ weights for the thyroid, T4 levels, or incidence of thyroid tumors. However, the presence of the histopathological finding for the thyroid in the low dose group and the increased incidence above the controls in the higher dose groups precludes the identification of a NOAEL for females.

There were microscopic pathology findings that may be of interest for other studies of Ziram toxicity. There was an increased incidence of foci of axonal degeneration in the spinal cord and sciatic nerve. It will be of interest to note if similar changes are seen in neurotoxicological studies for Ziram. Also of interest were the presence of prominent ultimobranchial cysts in the thyroid. These structures are considered to be of embryonic origin, thus it is possible that Ziram treatment may affect developing tissues. It may be of interest to note if similar changes are found in a developmental toxicity study for Ziram.

There was an increased incidence of benign tumors (hemangioma) in the spleen (1 male) and in the mesenteric lymph nodes (5 males) for males treated with Ziram at the MTD. The incidence of the neoplastic change for the mesenteric lymph nodes was increased statistically significantly ($p<0.05$) as compared to controls, and the incidence in this study was higher than in previous studies at this testing facility. The hemangioma showed no evidence of malignancy. These tumors were not found in females, in males in the medium or low dose groups, or in controls. There were no malignant tumors attributable to Ziram treatment. Dosing for males and females was adequate. The MTD for oral administration of Ziram to males and females for 104 weeks is 540 ppm, based upon the changes in body weights, food consumption, hematology, organ weights, and macroscopic and microscopic pathology.

E. STUDY DEFICIENCIES

A NOAEL could not be identified for males or females (see Discussion section above). The lack of a definitive NOAEL will require supplementary studies to be performed, even though a valid attempt was made to define the limit in the current study, MRID No. 434042-01. Creatinine phosphokinase and lactate dehydrogenase levels were not determined in the clinical chemistry evaluations. The appearance of urine or how urine was collected was not mentioned in the urinalysis section. The addition of data concerning these parameters would not change the conclusions, or the identification of the MTD.

Dose Selection Study in Rats

MRID No.:	424503-01
Study Type:	Subchronic Feeding-Rat (§82-1a), use for range-finding
Test Material:	Ziram
Study No.:	ZIR 5/901840
Sponsor:	Ziram Task Force, c/o UCB Chemicals Corporation, 5505-A Robin Hood Road, Norfolk, VA 23513 USA
Testing Facility:	Huntingdon Research Centre Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England
Study Title:	Preliminary toxicity to rats by dietary administration for 13 weeks.
Authors:	Lindsey A. J. Powell, David Crook, Richard Gregson, Chirukandath Gopinath, William A. Gibson, Alan Anderson
Study Completed:	August 19, 1992

Methods:

Test Animals:	Rats, Crl:CD(SD)BR, 42 days, male 157-188 g, female 117-144 g
Group Size:	10 males, 10 females per dose group
Test material concentrations:	Daily diet of Ziram at 0, 100, 300, or 1000 ppm.

Results:

Clinical signs: Increased incidence of hair loss in rats treated with Ziram at 300 and 1000 ppm. This was not noted for controls or rats treated with Ziram at 100 ppm.

Mortality: One female in the control group died during scheduled blood withdrawal. The cause of death is unknown.

Bodyweight: There were statistically significant ($p<0.01$), dose-dependent reductions in body weight gain for males and females treated with Ziram at 300 and 1000 ppm. Body weight gain for rats treated with Ziram at 100 ppm was similar to controls.

Food Consumption:

Food consumption was dose-dependently decreased for males and females. The decreases were statistically significant ($p<0.01$) for males and females treated with Ziram at 300 and 1000 ppm.

Clinical Pathology:

Hematology:

RBC and MCHC levels were dose-dependently decreased for both males and females. The decrease in RBC was statistically significant for males ($p<0.05$) and females ($p<0.01$) treated with Ziram at 1000 ppm. The decrease in MCHC was statistically significant for females at 100 ppm ($p<0.05$), and for males and females at 300 ($p<0.05$) and at 1000 ppm ($p<0.01$).

TABLE 13

Neoplastic morphology incidence summary - Main groups - male

Males on study	Group 1 70		Group 2 70		Group 3 70		Group 4 70	
Animals completed	Decedent 19	Terminal 31	Decedent 22	Terminal 28	Decedent 27	Terminal 23	Decedent 19	Terminal 31
Lymphoid/multicentric Tumours								
Examined	0	0	4	0	2	0	1	0
Follicular centre cell lymphoma (Malignant)	0	0	1	0	0	0	0	0
Histiocytic sarcoma (Malignant)	0	0	3	0	0	0	0	0
Lymphoid leukaemia (Malignant)	0	0	0	0	1	0	0	0
Myeloid leukaemia (Malignant)	0	0	0	0	1	0	0	0
Pleomorphic lymphoma (Malignant)	0	0	0	0	0	0	1	0
Heart								
Examined	19	31	22	2	27	1	19	31
Cardiac rhabdomyosarcoma (Malignant)	0	0	0	0	1	0	0	0
Lymph Nodes - Mesenteric								
Examined	19	31	22	28	27	23	19	31
Haemangioma (Benign)	0	0	0	0	0	0	0	5
Spleen								
Examined	19	31	22	28	27	23	19	31
Haemangioma (Benign)	0	0	0	0	0	0	1	0
Liver								
Examined	19	31	22	28	27	23	19	31
Hepatocellular adenoma (Benign)	0	1	0	1	0	1	0	2
Hepatocellular carcinoma (Malignant)	1	1	0	0	0	1	0	0
Pancreas								
Examined	19	31	22	28	27	23	19	31
Islet cell adenoma (Benign)	1	7	2	5	3	4	1	4
Islet cell carcinoma (Malignant)	1	0	1	1	2	1	0	0
Kidneys								
Examined	19	31	22	28	27	23	19	31

Appendix 2

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TABLE 13

(Neoplastic morphology incidence summary - male - continued)

Males on study	Group 1		Group 2		Group 3		Group 4	
	70		70		70		70	
Animals completed	Decedent 19	Terminal 31	Decedent 22	Terminal 28	Decedent 27	Terminal 23	Decedent 19	Terminal 31
Skeletal Muscle	(continued)							
Rhabdomyosarcoma (Malignant)	1	0	1	0	0	0	0	0
Fibrosarcoma (Malignant)	1	0	0	0	0	0	0	0
Fibroma (Benign)	0	0	0	0	0	0	0	1
Caecum								
Examined	19	31	22	1	27	2	19	31
Fibroma (Benign)	0	0	0	0	0	1	0	0
Skin								
Examined	19	31	22	10	27	8	19	31
Squamous cell papilloma (Benign)	1	0	0	0	0	0	0	0
Inverted squamous cell papilloma (Benign)	0	0	0	1	0	0	0	0
Sebaceous adenoma (Benign)	0	0	0	0	0	1	0	0
Basal cell carcinoma (Malignant)	0	0	1	0	0	0	0	0
Fibroma (Benign)	0	0	0	0	0	0	0	1
Dermal fibroma (Benign)	0	1	1	1	0	0	0	1
Subcutis								
Examined	3	4	8	4	5	7	4	8
Fibroma (Benign)	1	0	2	3	0	3	2	3
Myxofibroma (Benign)	1	1	0	0	1	0	0	1
Fibrosarcoma (Malignant)	1	1	0	0	0	0	1	1
Lipoma (Benign)	0	2	4	1	2	1	0	4
Basal cell tumour (Benign)	0	0	0	0	0	1	0	0
Mammary Glands								
Examined	19	31	22	1	27	2	19	31
Mammary fibroadenoma (Benign)	0	0	0	0	0	1	0	0
Mammary adenocarcinoma (Malignant)	0	0	1	0	0	0	0	0
Brain								
Examined	19	31	22	7	27	4	19	31

TABLE 13

Neoplastic morphology incidence summary - Main groups - females

Females on study	Group 1		Group 2		Group 3		Group 4	
	70		70		70		70	
Animals completed	Decedent 34	Terminal 16	Decedent 29	Terminal 21	Decedent 25	Terminal 25	Decedent 26	Terminal 24
Lymphoid/multicentric Tumours								
Examined	1	0	1	1	2	1	0	1
Histiocytic sarcoma (Malignant)	1	0	1	1	2	0	0	1
Malignant lymphoma (Malignant)	0	0	0	0	0	1	0	0
Spleen								
Examined	34	16	29	21	25	25	26	24
Haemangiosarcoma (Malignant)	1	0	0	0	0	0	0	0
Liver								
Examined	34	16	29	21	25	25	26	24
Hepatocellular adenoma (Benign)	1	0	0	0	1	2	0	0
Hepatocellular carcinoma (Malignant)	0	0	1	0	0	0	0	0
Pancreas								
Examined	34	16	29	21	24	25	26	24
Missing	0	0	0	0	1	0	0	0
Islet cell adenoma (Benign)	0	0	0	1	1	0	0	0
Kidneys								
Examined	34	16	29	21	25	25	26	24
Renal mesenchymal tumour (Malignant)	0	0	0	0	1	0	0	0
Ovaries								
Examined	34	16	29	21	25	25	25	24
Missing	0	0	0	0	0	0	1	0
Tubular adenoma (Benign)	0	0	0	1	0	0	0	0
Thecal cell tumour (Benign)	0	0	1	0	0	0	0	0
Uterus								
Examined	34	16	29	11	25	13	26	24
Leiomyosarcoma (Malignant)	0	0	0	0	2	0	0	0

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TABLE 13

(Neoplastic morphology incidence summary - Main groups - females - continued)

Females on study	Group 1		Group 2		Group 3		Group 4	
	70		70		70		70	
Animals completed	Decedent 34	Terminal 16	Decedent 29	Terminal 21	Decedent 25	Terminal 25	Decedent 26	Terminal 24
Mammary Glands	(continued)							
Mammary adenoma (Benign)	5	4	0	1	1	2	3	1
Mammary adenoma with epithelial atypia (Benign)	2	0	0	1	0	0	0	0
Mammary fibroadenoma (Benign)	15	7	15	10	5	16	10	13
Mammary fibroadenoma with epithelial atypia (Benign)	1	0	0	1	2	0	0	1
Mammary fibroma (Benign)	0	1	0	1	1	0	0	0
Mammary adenocarcinoma (Malignant)	3	1	1	3	5	3	0	1
Spinal Cord								
Examined	34	16	29	21	25	25	26	24
Astrocytoma (Malignant)	0	0	0	1	0	0	0	0
Brain								
Examined	34	16	29	12	25	7	26	24
Astrocytoma (Malignant)	0	1	0	0	0	0	0	0
Bone								
Examined	0	0	1	0	0	0	0	0
Osteosarcoma (Malignant)	0	0	1	0	0	0	0	0
Head								
Examined	1	1	1	1	0	5	0	0
Squamous cell carcinoma of Zymbal's gland (Malignant)	1	0	0	0	0	0	0	0

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Attachment 2

DATA EVALUATION REPORT

ZIRAM

Study Type: CHRONIC FEEDING/ONCOGENICITY- RAT (83-5)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Disclaimer

This final Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Oak Ridge National Laboratory personnel.

*Managed by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under Contract No. DE-AC05-84OR21400

There was no excess mortality in any of the treated groups relative to controls. Group mean body weight gains were decreased for males (86% of control, $p<0.01$ and females (74% of control, $p<0.01$) in the high dose group (540 ppm). Food consumption was decreased compared to controls for males (540 ppm: 91%, $p<0.01$) and females (180 ppm: 92%, $p<0.05$; 540 ppm: 94%, $p<0.05$). Hematology parameters (RBC, HGB, and PCV) were decreased relative to controls for females in the 540 ppm (weeks 26-104, $p<0.05$, $p<0.01$) and 180 ppm (weeks 26-52, $p<0.05$, $p<0.01$)) dose groups. There were statistically significant decreases ($p<0.05$, $p<0.01$) in clinical chemistry parameters (calcium, total protein, albumin, calcium and SGPT) during weeks 13-52 for females. For males (540 ppm, week 104) organ weight for the adrenals was decreased (absolute, 59% of control, $p<0.01$; relative, 67% of control, $p<0.05$). There were macroscopic pathological findings (not statistically significant) for animals in the 180 and 540 ppm dose groups for the stomach and skeletal muscle (males and females), and the adrenals (females only). There were microscopic pathological findings for males and females in the 180 and 540 ppm dose groups for spleen ($p<0.01$), liver ($p<0.01$, $p<0.05$), stomach ($p<0.05$, $p<0.01$), thyroid ($p<0.01$, $p<0.05$), skeletal muscle ($p<0.01$), spinal cord (males only, $p<0.05$), sciatic nerve (females only, $p<0.01$), and adrenal cortex ($p<0.05$, $p<0.01$). As there were histopathological findings for males in the 60 ppm dose group for spleen ($p<0.01$), stomach ($p<0.01$, $p<0.05$), skeletal muscle ($p<0.05$), and adrenal cortex ($p<0.05$), a NOAEL for males could not be identified. For females, there was an increase in prominent ultimobranchial cysts in the thyroid in all dose groups (Controls: 3/50; 60 ppm: 12/50, $p<0.05$; 180 ppm: 22/50, $p<0.01$;, 540 ppm: 27/50, $p<0.01$), precluding the identification of a NOAEL for females. **The NOAEL could not be identified for either males or females, due to histopathological findings for animals in the low dose group (60 ppm).**

Carcinogenic potential was evidenced by the finding of treatment-related tumors (benign hemangioma) in mesenteric lymph nodes (5/50, $p<0.05$) and in spleen (1/50) in males in the 540 ppm dose group. There were no treatment-related tumors identified in males in the 180 or 60 ppm dose groups, or in females in any dose group. There were no treatment-related malignant tumors in either sex. The dosing is adequate. Treatment of males with Ziram for 104 weeks at the MTD resulted in neoplastic changes.

This study is classified as Acceptable and satisfies the guideline requirements for a chronic/oncogenicity study (§83-5). This study did not establish a NOAEL.

Special Review Criteria (40 CFR 154.7) None

hematologic and clinical chemistry changes for the low dose group (100 ppm). There were effects on weight gain and food intake and minor hematological, clinical chemistry, organ weight and pathological changes for the medium and high dose groups (300 and 1000 ppm, respectively). The low dose for the current study was chosen to be 60 ppm, in an attempt to provide a NOEL. The medium and high doses were set at 3 and 9 times this dose (180 and 540 ppm, respectively).

TABLE 1. STUDY DESIGN

Dose Group	Doses (mg/kg/day)			No. Animals	
	Target Dose Both Sexes	Dosage Achieved (Mean[a])		Male	Female
		Males	Females		
1 Control[b]	0 ppm	0	0	50	50
2 Low (LDT)[b]	60 ppm[c]	2.5	3.4	50	50
3 Mid (MDT)[b]	180 ppm	7.7	10.2	50	50
4 High (HDT)[b]	540 ppm	23.7	34.6	50	50
5 Satellite Control[d]	0 ppm	0	0	20	20
6 Satellite (LDT)[d]	60 ppm[c]	3.0	3.9	20	20
7 Satellite (MDT)[d]	180 ppm	9.1	11.7	20	20
8 Satellite (HDT)[d]	540 ppm	27.3	37.5	20	20

Data obtained from summary table on p. 43, MRID No. 43404201.
[a]Compound intake was calculated by the study authors on a weekly basis. The mean of the weekly values over the 104 week test period is presented.
[b]Data for weeks 1-104: includes combined data for main and satellite groups during weeks 1-52 and data for main group during weeks 53-104.
[c]Diet prepared contained 66 ppm.
[d]Achieved dose for satellite groups alone was not presented in the study report (MRID No. 434042-01). Data was combined by the study authors for satellite and main groups for weeks 1-52

2. Diet preparation and analysis

Diet was prepared weekly. A concentrate was prepared by grinding appropriate amounts of Ziram with untreated sieved basal diet and mixing in a Turbula mixer for at least 2 minutes. The concentrated diet was diluted with appropriate quantities of untreated diet and homogeneity was achieved by mixing in a double-cone blender for at least 7 minutes. The total volume of diet required was large, such that the diets for each dose were prepared in 2 batches. Trial studies evaluating the stability of Ziram in rodent diet formulations showed that the compound was not stable at low doses (70 ppm) when stored at ambient temperature,

following sequence. Invalid values (documented by machine fault, organ loss or damage) are excluded. If the relative frequency of the mode is greater than 75%, the proportion of animals with values different from the mode were analyzed. Otherwise, Bartlett's test was used for analysis of heterogeneity of variance between treatments. If there was significant (1% level) heterogeneity, the data were transformed logarithmically. If no significant heterogeneity was found, or if the data transformation was satisfactory, a one-way analysis of variance was performed. If significant heterogeneity of variance was present and could not be removed by logarithmic transformation, the Kruskal-Wallis' analysis of ranks was used. Analyses of variance were followed by Student's *t*-test and Williams' test for a dose-related response. Kruskal-Wallis' test was followed by non-parametric equivalents of the *t*-test and Williams' test (Shirley's test). Where appropriate, an analysis of covariance was used in place of an analysis of variance. For organ weight data, an analysis of variance was performed using terminal body weight as a covariate when the within-group relationship between organ weight and body weight was significant at the 10% level. Mortality was analyzed using log rank methods. Incidence of tumors was analyzed according to the context of the observation as interpreted by the pathologist. Trend tests were used based upon nominal dose levels.

5. Signed and dated GLP/quality assurance statements were present.

C. METHODS AND RESULTS

1. Observations

Animals were palpated and inspected daily during the first 4 weeks and once a week thereafter for signs of behavioral changes, reactions to treatment, and ill health. Checks for dead and/or moribund animals were performed twice daily.

Results – There were no clinical signs indicative of a response to treatment. The mortality distribution did not indicate an adverse effect of Ziram treatment (Table 2). Mortality for females in the 180 (p=0.035) and 540 (p=0.029) ppm dose groups was statistically significantly decreased relative to controls. This was likely due to decreased survival for the control group (32%, expected 50% at 104 weeks). The overall test for trend was not significant.

TABLE 3. GROUP MEAN BODY WEIGHTS (G/RAT) AT SELECTED WEEKS & GROUP MEAN BODY WEIGHT CHANGES (G/RAT) AT SELECTED WEEKLY INTERVALS

Week of Study	Males				Females			
	0	60[a]	180	540	0	60[a]	180	540
0	174	173	174	174	142	142	142	144
1	222	219	208	186	168	168	163	150
4	332	331	318	285	220	220	214	193
13	485	490	477	428	282	283	275	252
26	597	607	584	522	332	333	311	287
52	707	730	694	620	426	424	383	338
104	752	740	697	671	470	466	465	388
Weeks 0-1	48.4	45.9 (95%[b])	34.1** (70%)	12.2** (25%)	25.6	26.0 (102%)	20.6** (80%)	5.8** (23%)
Weeks 0-52	534	557 (104%)	520 (97%)	446** (84%)	284	282 (99%)	241** (85%)	194** (68%)
Weeks 1-104	528	520 (98%)	489 (93%)	486 (92%)	304	303 (100%)	302 (99%)	238* (78%)
Weeks 0-104	576	565 (98%)	523 (91%)	496** (86%)	329	326 (99%)	323 (98%)	245** (74%)

Data adapted from summary tables, pp. 39-40 and Table 2, pp. 72-75, Appendix 2, pp. 303-446, MRID No. 42450301.
[a]Diet prepared to contain 66 ppm.
[b]% of control
*p < 0.05, **p < 0.01, Data for weeks 0-52 included both main and satellite group animals. For statistical analysis data for females for weeks 0-52 were log-transformed. For weeks 0-1, 1-104, and 0-104 (males), Kruskal-Wallis analysis of mean ranks was applied.

3. Food consumption and compound intake

Food consumption for each cage was determined daily and reported as food intake (g) per rat per week, based upon the number of surviving animals in the cage. Mean daily diet consumption was calculated by the reviewer as g food/kg body weight/day. Food efficiency (body weight gain, kg/food consumption, kg per unit time X 100) was calculated by the reviewer. Compound intake (mg/kg/day) values were calculated as time-weighted averages from the food consumption and mid-week body weight data.

Results –

a. Food consumption – Food consumption (Table 4) was statistically signficantly decreased for males in the 540 ppm dose group (91% of control, $p<0.01$) and females in the 180 (92% of control, $p<0.05$) and 540 ppm dose groups (94% of control, $p<0.05$). Food consumption for rats treated with Ziram at 60 ppm was similar to controls.

5. Blood was collected during weeks 13, 26, 52, 78, and at termination under ether anesthesia and after overnight fast from the orbital sinus of 10 male and 10 female rats from each dose group for hematology and clinical analysis. Rats from the satellite group were used for samples obtained in weeks 13, 26, and 52. Rats from the main group were used for the week 78 and termination samples. Where possible, the same rats were used for drawing blood. In week 4, blood samples were withdrawn from the orbital sinus of 10 male and 10 female rats for T3, T4, and TSH measurements. Blood samples were collected and mixed with EDTA for hematology, citrate for blood coagulation tests, or heparin for clinical chemistry evaluations. The CHECKED (X) parameters were examined.

a. Hematology

X			X		
X	Hematocrit (HCT)*		X	Leukocyte differential count*	
X	Hemoglobin (HGB)*		X	Mean corpusc. HGB conc. (MCHC)	
X	Leukocyte count (WBC)*		X	Mean corpusc. volume (MCV)	
X	Erythrocyte count (RBC)*			Reticulocyte count	
X	Platelet count*				
X	Blood clotting measurements				
	(Thromboplastin time)				
X	(Clotting time)				
	(Prothrombin time)				

* Required for subchronic and chronic studies.

Results – There were changes in the hematological parameters RBC, HCT, HGB, MCHC, MCV, and clotting time that were statistically significantly different from control values (Table 5, $p<0.05$ and $p<0.01$). The most consistent change was decreased RBC levels relative to controls. The decreases in RBC were evident for males (180, 540 ppm) at week 13 and for females treated with Ziram at 180 ppm at weeks 26 and 52 and at 540 ppm from week 26 to 104. HGB and PCV values were correspondingly decreased at most of these timepoints and the decreases were generally statistically significant ($p<0.05$, $p<0.01$). The values for all of the hematological parameters fell within the historical control range established by the testing facility. The only statistically significant change for animals treated with Ziram at 60 ppm was a decreased blood clotting time for males at week 26 ($p<0.05$).

b. Clinical chemistry

X		X	
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
Enzymes		X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatinine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	Tri-iodothyronine (T3)
X	Serum alanine aminotransferase (also SGPT)*	X	Thyroxine (T4)
X	Serum aspartate aminotransferase (also SGOT)*	X	Thyroid Stimulating Hormone (TSH)
X	Glutamic-oxaloacetic transaminase (GOT)		
	Glutamate dehydrogenase		

* Required for subchronic and chronic studies.

Results – For males (Table 6), there were statistically significant decreases relative to controls in total serum protein (94-98.5% of control, $p \leq 0.05$), albumin (93-96.7% of control, $p \leq 0.05$, $p \leq 0.01$), SGPT (66.7-77% of control, $p \leq 0.05$, $p \leq 0.01$), calcium (96-98% of control, $p \leq 0.05$, $p \leq 0.01$), and T4 (75.7-85% of control, $p \leq 0.05$, $p \leq 0.01$) levels and increases relative to controls in urea (115-121% of control, $p \leq 0.05$) and ALK (125-142% of control, $p \leq 0.05$) levels. For females, there were statistically significant decreases relative to controls in total serum protein (93-95.7% of control, $p \leq 0.05$, $p \leq 0.01$), albumin (91-88.6% of control, $p \leq 0.05$, $p \leq 0.01$), SGPT (43-44% of control, $p \leq 0.05$, $p \leq 0.01$), SGOT (26% of control, $p \leq 0.05$), calcium (94-96% of control, $p \leq 0.05$, $p \leq 0.01$), and T4 (74% of control, $p \leq 0.05$) levels and increases in urea (142-143% of control, $p \leq 0.01$), chloride (101-102% of control, $p \leq 0.05$, $p \leq 0.01$) levels. The increases in ALK levels were dose-dependent for both males and females. There was a dose-dependent decrease in SGOT levels for females at weeks 26 and 52. SGOT levels for females in the control and 60 ppm dose groups were higher than males in these dose groups at weeks 26 and 52. Thus, SGOT levels for females in the 540 ppm dose group at week 26 were statistically significantly (26% of control, $p \leq 0.05$) lower than controls. The only statistically significant changes affecting rats in the 60 ppm dose group were a decrease in total protein levels for males at week 52, relative to control (97.1% of control, $p \leq 0.05$), and an increase in chloride levels for females at week 13, relative to control (101% of control, $p \leq 0.05$).

TABLE 6 (Continued)

Parameter	Week of Study	Exposure Level (ppm)							
		Males (N=10)				Females (N=10)			
		0	60*	180	540	0	60*	180	540
SGOT	13	54	54	51	52	82	62	57	53
	26	50	55	51	50	207	102	77	53*
	52	56	55	56	53	159	84	75	71
	78	53	61	59	45	77	73	90	63
	104	51	55	75	50	77	78	97	80
Calcium	13	5.4	5.5	5.4	5.2**	5.4	5.4	5.3*	5.1**
	26	5.5	5.5	5.5	5.4*	5.6	5.6	5.5	5.4**
	52	5.6	5.6	5.5	5.5	5.6	5.6	5.7	5.4**
	78	5.5	5.4	5.4**	5.4**	5.4	5.6	5.4	5.4
	104	5.4	5.4	5.3	5.3	5.4	5.6	5.3	5.3
Chloride	13	101	101	102	100	101	102*	103*	102*
	26	101	101	100	100	100	101	102**	102**
	52	100	102	101	101	99	100	100	101*
	78	102	103	102	102	99	100	100	101*
	104	100	100	100	101	98	96	97	98
T4	4	3.7	3.4	3.0**	2.8**	2.7	2.4	2.4	2.0*
	13	3.7	3.4	3.2	3.2	2.9	2.7	2.7	2.6
	26	3.4	3.3	3.1	2.9*	2.3	2.4	2.5	2.4

Data taken from summary table, p. 47, MRID No. 42434001. Parameter unit are: Total serum protein (g/dL), Albumin (g/dL), Urea (blood urea nitrogen, mg/dL), ALK (mU/mL), SGPT (mU/mL), SGOT (mU/mL), Ca^{+} (mEq/L), Cl^{-} (mEq/L), T4 (μg/dL).
*Diet prepared to contain 66 ppm.
*$p \leq 0.05$
**$p \leq 0.01$

6. Urinalysis

Urine was collected in an unspecified manner from 10 male and 10 female animals per dose group during weeks 13, 26, 52 (satellite group), 78, and at termination (main group). Food and water were removed during the overnight collection period (~16 hours). The CHECKED (X) parameters were examined.

TABLE 7. SUMMARY OF STATISTICALLY SIGNIFICANT CHANGES IN URINALYSIS PARAMETERS IN RATS ADMINISTERED ZIRAM FOR 104 WEEKS.

Parameter	Week of Study	Treatment Group/Exposure Level (ppm)							
		Males (N=10)				Females (N=10)			
		0	60[a]	180	540	0	60[a]	180	540
*p*H	13	7.0	6.9	7.3	7.5**	6.4	6.4	6.5	6.5
	26	6.9	7.1	7.6**	7.7*	6.5	6.6	6.5	6.6
	52	6.8	6.9	7.3*	7.3*	6.2	6.4	6.4	6.3
	78	6.9	7.1	7.0	7.4*	6.2	6.4	6.3	6.6*
	104	6.4	6.6	6.2	6.7	6.3	6.2	6.4	6.4
Volume	13	6.2	6.1	6.0	5.6	2.6	3.1	2.7	2.0
	26	5.1	6.7	6.9	6.9	3.2	3.1	2.5	3.2
	52	6.3	7.1	5.9	5.9	6.6	5.1	5.6	3.5*
	78	10.6	10.4	9.0	7.6	6.6	8.2	8.4	6.9
	104	9.4	7.1	10.4	8.8	10.7	8.3	12.9	10.6
Protein	13	175	171	188	168	81	90	82	75
	26	201	177	135	132	73	83	70	66
	52	272	220	215	334	81	142	134	87
	78	336	116	188	114	518	107	100	100
	104	552	289	929	477	337	652	200	73*

Data taken from Table 9, pp. 107-111, MRID No. 42434001. Parameter units are: Volume (mL), Protein (mg/dL).
[a]Diet prepared to contain 66 ppm.
*$p \leq 0.05$
**$p \leq 0.01$

Results –

a. Organ weight – For males (Table 8), absolute organ weight for adrenals were dose-dependently decreased at weeks 52 and 104. The decrease was statistically significant at week 104 for males in the high dose group for absolute (58.8% of control, $p<0.01$) and relative (66.7% of control, $p<0.05$) adrenal weights. Relative brain weights for males in the 540 ppm dose group were statistically significantly increased at week 104 (110% of control, $p<0.05$). Relative testes weights were statistically significantly increased at week 52 for males in the 540 ppm dose group (115% of control, $p<0.05$) and at week 104 for males in the 180 (112.5% of control, $p<0.05$) and 540 ppm (127% of control, $p<0.01$) dose groups. Relative organ weights were statistically significantly increased for females in the high dose group relative to controls for brain (weeks 52 and 104, 120% of control, $p<0.01$), thyroid (week 104, 133% of control, $p<0.05$), heart (week 52, 117% of control, $p<0.01$; week 104, 112% of control, $p<0.05$), kidney (week 52, 111% of control, $p<0.05$), and adrenal (week 104, 156% of control, $p<0.01$). For females, relative liver weights were dose-dependently increased at weeks 52 and 104. The increases were statistically significant at week 52 for females in the 60, 180, and 540 ppm dose groups (113%, 113%, and 120% of control, respectively, $p<0.01$) and at week 104 for females in the 540 ppm dose group (112% of control, $p<0.05$). Relative pituitary weights were statistically significantly decreased (72.5% of control, $p<0.05$) at week 52 for females in the 540 ppm dose group.

b. Gross pathology – Treatment related findings (Table 9) occurring at a higher incidence in treated animals than in controls included depressions, raised areas, thickening, and white discoloration in the forestomach, cysts and cystic enlargement in adrenals (females only), and atrophy of hindlimbs. The incidence of these findings were not statistically significantly different from controls, however, there are correlates with microscopic pathological findings for stomach, adrenals, skeletal muscle, spinal cord, and sciatic nerve. There was also an increased incidence of absence of corpora lutea for females treated with Ziram at 60, 180, and 540 ppm (44%, 48%, 62%, respectively) as compared to controls (38%). This finding is not likely toxicologically significant. In the microscopic pathological examination of the ovaries, there was a similar incidence of absence of corpora lutea in treated animals as compared to controls (see Table 10 of this report).

TABLE 9. MACROSCOPIC PATHOLOGY INCIDENCE SUMMARY FOR RATS ADMINISTERED ZIRAM FOR 104 WEEKS.

Pathology	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	60	180	540	0	60	180	540
No. animals	50	50	50	50	50	50	50	50
Ovaries								
-no corpora lutea visible	---	---	---	---	19	22	24	31
Forestomach								
-Depression	11	16	20	20	8	7	14	31
-Raised Areas	0	0	3	4	0	1	1	0
-Thickening	5	11	11	12	3	3	8	12
-White Discoloration	4	8	7	6	1	1	4	9
Overall Pathology Incidence for Forestomach	20	35	41	42	12	12	27	52
Adrenals								
-Cysts/Cystic	0	0	0	0	0	0	1	5
-Cystic enlargement	0	0	0	0	0	0	0	2
Skeletal muscle								
-Atrophied hindlimbs	4	5	3	6	0	0	0	2

Data adapted from summary table, p. 50, MRID No. 42434001.

TABLE 10. MICROSCOPIC PATHOLOGY: NON-NEOPLASTIC CHANGES FOR MAIN GROUP RATS ADMINISTERED ZIRAM FOR 104 WEEKS

Pathology	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	60	180	540	0	60	180	540
No. animals (main group)	50	50	50	50	50	50	50	50
Spleen								
No abnormalities detected	22	13*	13*	14	14	9	2**	3**
Hemosiderosis	10 (2.3)[a]	22** (1.6)	29** (2.0)	23** (2.0)	24 (2.8)	29 (2.2)	38** (2.3)	39** (2.9)
Liver								
Pigment (hemosiderin) in sinusoidal cells	2 (1.5)	5 (1.2)	22** (1.3)	26** (1.4)	0 (0)	3 (1.3)	13** (1.8)	15** (1.9)
Bile duct hyperplasia	7 (1.9)	7 (2.0)	13 (2.1)	15* (2.1)	5 (1.8)	6 (1.8)	9 (2.1)	17** (2.1)
Stomach-Non-Glandular Region								
No abnormalities detected	31	28	18**	20*	32	35	27	11**
Epithelial hyperplasia	6 (2.5)	18** (2.7)	25** (2.6)	25** (2.7)	7 (2.3)	6 (2.5)	15* (2.5)	37** (2.5)
Subepithelial edema	2 (3.0)	8* (2.75)	8* (2.75)	10* (2.7)	2 (2.5)	3 (3.3)	3 (2.3)	11** (2.7)
Ulceration	5 (2.4)	8 (2.4)	14* (2.4)	14* (2.4)	3 (2.3)	5 (2.8)	6 (2.5)	12* (2.2)
Perforating ulceration, marked	0	1	0	3	0	0	0	1
Hyperplasia at the limiting ridge	1 (2.0)	2 (2.0)	3 (2.0)	3 (2.3)	1 (2.0)	2 (2.5)	4 (2.8)	1 (2.0)
Pancreas								
Replacement by adipose tissue	7 (2.4)	14 (2.4)	15* (2.3)	21** (2.7)	0/50 (0)	2/50 (1.5)	4/49 (2.0)	3/50 (2.7)
Thyroid								
No abnormalities detected	29	31	25	24	37	31	20**	17**
C-cell hyperplasia	1 (2.0)	3 (2.3)	5 (2.2)	8* (2.5)	5 (2.8)	5 (2.2)	5 (2.2)	3 (2.3)
Prominent ultimobranchial cysts	4	5	14**	15**	3	12*	22**	27**
Parathyroids								
No abnormalities detected	48/49	40*/47	44/48	39*/46	47/48	46/46	46/47	47/47
Hyperplasia	1/49 (2.0)	4/47 (2.5)	4/48 (2.3)	7*/46 (2.3)	0	0	0	0

Appendix 2). The tumors with increased or decreased incidence relative to control are presented in Table 11. The incidence of males with tumors was decreased dose-dependently compared to controls (540 ppm, $p<0.05$). This decrease was primarily due to a dose-dependent decrease in malignant tumors for males (540 ppm, $p<0.01$). The only tumors considered by the study authors to be treatment-related were hemangiomas in mesenteric lymph nodes and spleens of males. These tumors were benign, showing no evidence of malignancy. The incidence of animals with this tumor type was statistically significantly increased for males treated with Ziram at 540 ppm ($p=0.024$), as compared to controls. In addition, the dose-related trend in the number of animals with this tumor type was statistically significant ($p=0.0001$). Historical control data obtained by the testing facility indicated that the incidence for this tumor type in males ranged from 0/50 to 2/50. The overall incidence for this tumor type in the current study was 6/50. For females, there were no tumors found with a higher incidence in treated animals than in controls that were considered by the study authors to be treatment-related. Appendix 2 summarizes tumor types and incidences in male and female rats of the main study groups.

TABLE 11. MICROSCOPIC PATHOLOGY: NEOPLASTIC CHANGES FOR MAIN GROUP RATS ADMINISTERED ZIRAM FOR 104 WEEKS

Neoplasia	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	60	180	540	0	60	180	540
No. animals	50	50	50	50	50	50	50	50
No. of animals with tumors[a]	45	44	41	37*	48	46	49	47
No. of animals with malignant tumors[a]	23	20	17	8**	12	15	21*	13
No. of animals with benign tumors[a]	35	34	32	35	46	41	42	43
No. of animals with metastatic tumors[a]	3	1	1	2	0	0	2	1
Lymph Nodes-Mesenteric								
Hemangioma (Benign)[b]	0	0	0	5*	0	0	0	0
Spleen								
Hemangioma (Benign)[b]	0	0	0	1	0	0	0	0

Data adapted from Table 13, p. 142-296, MRID No. 42434001.
[a]Statistical significance calculated by the reviewer, *t*-test.
[b]Statistical significance calculated by the study authors.
*$p<0.05$
**$p<0.01$

D. DISCUSSION

From the results presented in the current study report, MRID No. 434042-01, oral administration of Ziram at 60, 180, and 540 ppm for 104 weeks to male and female rats resulted in

bundles, $p<0.05$), and adrenal cortex (hypertrophy with vacuolation, $p<0.05$). These histopathologic changes were present in generally greater severity in the higher dose groups (180 and 540 ppm), were not present in controls, and thus are toxicologically significant. For females treated with Ziram at the low dose (60 ppm), the only treatment-related effect was a statistically significant increase ($p<0.05$) in the incidence of prominent ultimobranchial cysts in the thyroid. The toxicological significance of this finding is not clear. There were no statistically significant differences between controls and females treated with Ziram at 60 ppm in absolute or relative organ weights for the thyroid, T4 levels, or incidence of thyroid tumors. However, the presence of the histopathological finding for the thyroid in the low dose group and the increased incidence above the controls in the higher dose groups precludes the identification of a NOAEL for females.

There were microscopic pathology findings that may be of interest for other studies of Ziram toxicity. There was an increased incidence of foci of axonal degeneration in the spinal cord and sciatic nerve. It will be of interest to note if similar changes are seen in neurotoxicological studies for Ziram. Also of interest were the presence of prominent ultimobranchial cysts in the thyroid. These structures are considered to be of embryonic origin, thus it is possible that Ziram treatment may affect developing tissues. It may be of interest to note if similar changes are found in a developmental toxicity study for Ziram.

There was an increased incidence of benign tumors (hemangioma) in the spleen (1 male) and in the mesenteric lymph nodes (5 males) for males treated with Ziram at the MTD. The incidence of the neoplastic change for the mesenteric lymph nodes was increased statistically significantly ($p<0.05$) as compared to controls, and the incidence in this study was higher than in previous studies at this testing facility. The hemangioma showed no evidence of malignancy. These tumors were not found in females, in males in the medium or low dose groups, or in controls. There were no malignant tumors attributable to Ziram treatment. Dosing for males and females was adequate. The MTD for oral administration of Ziram to males and females for 104 weeks is 540 ppm, based upon the changes in body weights, food consumption, hematology, organ weights, and macroscopic and microscopic pathology.

E. STUDY DEFICIENCIES

A NOAEL could not be identified for males or females (see Discussion section above). The lack of a definitive NOAEL will require supplementary studies to be performed, even though a valid attempt was made to define the limit in the current study, MRID No. 434042-01. Creatinine phosphokinase and lactate dehydrogenase levels were not determined in the clinical chemistry evaluations. The appearance of urine or how urine was collected was not mentioned in the urinalysis section. The addition of data concerning these parameters would not change the conclusions, or the identification of the MTD.

Dose Selection Study in Rats

MRID No.:	424503-01
Study Type:	Subchronic Feeding-Rat (§82-1a), use for range-finding
Test Material:	Ziram
Study No.:	ZIR 5/901840
Sponsor:	Ziram Task Force, c/o UCB Chemicals Corporation, 5505-A Robin Hood Road, Norfolk, VA 23513 USA
Testing Facility:	Huntingdon Research Centre Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England
Study Title:	Preliminary toxicity to rats by dietary administration for 13 weeks.
Authors:	Lindsey A. J. Powell, David Crook, Richard Gregson, Chirukandath Gopinath, William A. Gibson, Alan Anderson
Study Completed:	August 19, 1992

Methods:

Test Animals:	Rats, Crl:CD(SD)BR, 42 days, male 157-188 g, female 117-144 g
Group Size:	10 males, 10 females per dose group
Test material concentrations:	Daily diet of Ziram at 0, 100, 300, or 1000 ppm.

Results:

Clinical signs: Increased incidence of hair loss in rats treated with Ziram at 300 and 1000 ppm. This was not noted for controls or rats treated with Ziram at 100 ppm.

Mortality: One female in the control group died during scheduled blood withdrawal. The cause of death is unknown.

Bodyweight: There were statistically significant ($p<0.01$), dose-dependent reductions in body weight gain for males and females treated with Ziram at 300 and 1000 ppm. Body weight gain for rats treated with Ziram at 100 ppm was similar to controls.

Food Consumption:

Food consumption was dose-dependently decreased for males and females. The decreases were statistically significant ($p<0.01$) for males and females treated with Ziram at 300 and 1000 ppm.

Clinical Pathology:

Hematology:

RBC and MCHC levels were dose-dependently decreased for both males and females. The decrease in RBC was statistically significant for males ($p<0.05$) and females ($p<0.01$) treated with Ziram at 1000 ppm. The decrease in MCHC was statistically significant for females at 100 ppm ($p<0.05$), and for males and females at 300 ($p<0.05$) and at 1000 ppm ($p<0.01$).

TABLE 13

Neoplastic morphology incidence summary - Main groups - male

Males on study	Group 1		Group 2		Group 3		Group 4	
	70		70		70		70	
Animals completed	Decedent 19	Terminal 31	Decedent 22	Terminal 28	Decedent 27	Terminal 23	Decedent 19	Terminal 31
Lymphoid/multicentric Tumours								
Examined	0	0	4	0	2	0	1	0
Follicular centre cell lymphoma (Malignant)	0	0	1	0	0	0	0	0
Histiocytic sarcoma (Malignant)	0	0	3	0	0	0	0	0
Lymphoid leukaemia (Malignant)	0	0	0	0	1	0	0	0
Myeloid leukaemia (Malignant)	0	0	0	0	1	0	0	0
Pleomorphic lymphoma (Malignant)	0	0	0	0	0	0	1	0
Heart								
Examined	19	31	22	2	27	1	19	31
Cardiac rhabdomyosarcoma (Malignant)	0	0	0	0	1	0	0	0
Lymph Nodes - Mesenteric								
Examined	19	31	22	28	27	23	19	31
Haemangioma (Benign)	0	0	0	0	0	0	0	5
Spleen								
Examined	19	31	22	28	27	23	19	31
Haemangioma (Benign)	0	0	0	0	0	0	1	0
Liver								
Examined	19	31	22	28	27	23	19	31
Hepatocellular adenoma (Benign)	0	1	0	1	0	1	0	2
Hepatocellular carcinoma (Malignant)	1	1	0	0	0	1	0	0
Pancreas								
Examined	19	31	22	28	27	23	19	31
Islet cell adenoma (Benign)	1	7	2	5	3	4	1	4
Islet cell carcinoma (Malignant)	1	0	1	1	2	1	0	0
Kidneys								
Examined	19	31	22	28	27	23	19	31

TABLE 13

(Neoplastic morphology incidence summary - male - continued)

Males on study	Group 1		Group 2		Group 3		Group 4	
	70		70		70		70	
Animals completed	Decedent 19	Terminal 31	Decedent 22	Terminal 28	Decedent 27	Terminal 23	Decedent 19	Terminal 31
Skeletal Muscle	(continued)							
Rhabdomyosarcoma (Malignant)	1	0	1	0	0	0	0	0
Fibrosarcoma (Malignant)	1	0	0	0	0	0	0	0
Fibroma (Benign)	0	0	0	0	0	0	0	1
Caecum								
Examined	19	31	22	1	27	2	19	31
Fibroma (Benign)	0	0	0	0	0	1	0	0
Skin								
Examined	19	31	22	10	27	8	19	31
Squamous cell papilloma (Benign)	1	0	0	0	0	0	0	0
Inverted squamous cell papilloma (Benign)	0	0	0	1	0	0	0	0
Sebaceous adenoma (Benign)	0	0	0	0	0	1	0	0
Basal cell carcinoma (Malignant)	0	0	1	0	0	0	0	0
Fibroma (Benign)	0	0	0	0	0	0	0	1
Dermal fibroma (Benign)	0	1	1	1	0	0	0	1
Subcutis								
Examined	3	4	8	4	5	7	4	8
Fibroma (Benign)	1	0	2	3	0	3	2	3
Myxofibroma (Benign)	1	1	0	0	1	0	0	1
Fibrosarcoma (Malignant)	1	1	0	0	0	0	1	1
Lipoma (Benign)	0	2	4	1	2	1	0	4
Basal cell tumour (Benign)	0	0	0	0	0	1	0	0
Mammary Glands								
Examined	19	31	22	1	27	2	19	31
Mammary fibroadenoma (Benign)	0	0	0	0	0	1	0	0
Mammary adenocarcinoma (Malignant)	0	0	1	0	0	0	0	0
Brain								
Examined	19	31	22	7	27	4	19	31

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TABLE 13

Neoplastic morphology incidence summary - Main groups - females

	Group 1		Group 2		Group 3		Group 4	
Females on study	70		70		70		70	
Animals completed	Decedent 34	Terminal 16	Decedent 29	Terminal 21	Decedent 25	Terminal 25	Decedent 26	Terminal 24
Lymphoid/multicentric Tumours								
Examined	1	0	1	1	2	1	0	1
Histiocytic sarcoma (Malignant)	1	0	1	1	2	0	0	1
Malignant lymphoma (Malignant)	0	0	0	0	0	1	0	0
Spleen								
Examined	34	16	29	21	25	25	26	24
Haemangiosarcoma (Malignant)	1	0	0	0	0	0	0	0
Liver								
Examined	34	16	29	21	25	25	26	24
Hepatocellular adenoma (Benign)	1	0	0	0	1	2	0	0
Hepatocellular carcinoma (Malignant)	0	0	1	0	0	0	0	0
Pancreas								
Examined	34	16	29	21	24	25	26	24
Missing	0	0	0	0	1	0	0	0
Islet cell adenoma (Benign)	0	0	0	1	1	0	0	0
Kidneys								
Examined	34	16	29	21	25	25	26	24
Renal mesenchymal tumour (Malignant)	0	0	0	0	1	0	0	0
Ovaries								
Examined	34	16	29	21	25	25	25	24
Missing	0	0	0	0	0	0	1	0
Tubular adenoma (Benign)	0	0	0	1	0	0	0	0
Thecal cell tumour (Benign)	0	0	1	0	0	0	0	0
Uterus								
Examined	34	16	29	11	25	13	26	24
Leiomyosarcoma (Malignant)	0	0	0	0	2	0	0	0

TABLE 13

(Neoplastic morphology incidence summary - Main groups - females - continued)

Females on study	Group 1 70		Group 2 70		Group 3 70		Group 4 70	
Animals completed	Decedent 34	Terminal 16	Decedent 29	Terminal 21	Decedent 25	Terminal 25	Decedent 26	Terminal 24
Mammary Glands	(continued)							
Mammary adenoma (Benign)	5	4	0	1	1	2	3	1
Mammary adenoma with epithelial atypia (Benign)	2	0	0	1	0	0	0	0
Mammary fibroadenoma (Benign)	15	7	15	10	5	16	10	13
Mammary fibroadenoma with epithelial atypia (Benign)	1	0	0	1	2	0	0	1
Mammary fibroma (Benign)	0	1	0	1	1	0	0	0
Mammary adenocarcinoma (Malignant)	3	1	1	3	5	3	0	1
Spinal Cord								
Examined	34	16	29	21	25	25	26	24
Astrocytoma (Malignant)	0	0	0	1	0	0	0	0
Brain								
Examined	34	16	29	12	25	7	26	24
Astrocytoma (Malignant)	0	1	0	0	0	0	0	0
Bone								
Examined	0	0	1	0	0	0	0	0
Osteosarcoma (Malignant)	0	0	1	0	0	0	0	0
Head								
Examined	1	1	1	1	0	5	0	0
Squamous cell carcinoma of Zymbal's gland (Malignant)	1	0	0	0	0	0	0	0

Attachment 3

Spleen

A haemangioma was seen in the spleen of a single male decedent (animal 260) animal from the same 540 ppm dosage group (see table below).

Incidence of major neoplastic changes	Control		60† ppm		180 ppm		540 ppm	
	♂	♀	♂	♀	♂	♀	♂	♀
Haemangiomata								
Total	0	0	0	0	0	0	6*	0
Mesenteric nodes	0	0	0	0	0	0	5*	0
Spleen	0	0	0	0	0	0	1	0
Number of animals	50	50	50	50	50	50	50	50

NOTE: Statistical significance of lesion incidence in treated groups compared to controls were determined by Fisher's test: * $P \leq 0.05$

† Prepared to contain 66 ppm (+10%)

Haemangiomata were not observed in other tissues in these animals. They were not seen in any male rats receiving lower doses of Ziram, in any of the female rats, nor in control rats. A haemangiosarcoma was seen in the spleen of a single female intercurrent death animal of the control group. Angiectasis was not an associated finding.

Statistical analysis of the relative group incidence for this tumour type by the time-to-tumour methods recommended by the International Agency for Research on Cancer (IARC) demonstrated a statistically significant dose-related trend in the number of animals with tumours ($P=0.0001$) and a significant difference between the control and the 540 ppm dosage group ($P=0.024$). This analysis is presented in the **STATISTICAL ANALYSIS OF TUMOUR INCIDENCE** (page 2346).

Haemangiomata are benign multilocular tumours occasionally seen in low numbers in rats of this strain and age range, mesenteric lymph nodes and the spleen being the commonest sites for their detection. The incidence which was recorded in the 540 ppm dosage group of this study of some 5/50 animals in a single site (6/50 in total) is outside the normal background incidence for this laboratory where a range from 0/50 in the majority of instances to a maximum of 2/50 in control animals is recorded for a series of recent and concurrent studies utilising this strain of rat as shown in the following tabulation (male animals only):

Background incidence data for recent studies	89 01	89 02	89 03	89 04	89 05	89 06	89 07	89 08	89 09	89 10
Haemangiomata										
Lymph nodes	0	0	1	1	0	0	2	1	0	0
Spleen	0	0	0	0	0	0	0	0	0	0
Number of control animals	50	50	50	50	50	50	50	50	60	52

It is, therefore, considered that the presence of these haemangiomata was a consequence of the administration of Ziram at a dosage level of 540 ppm to male animals.

Attachment 4

MEMORANDUM August 25, 1999

SUBJECT: Ziram Qualitative Risk Assessment Based On Crl: CD(SD)BR Rat Dietary Study

P.C. Code 034805

TO: Patricia Gaunt, Toxicologist
Reregistration Branch 4
Health Effects Division (7509C)

FROM: Lori L. Brunsman, Statistician
Science Analysis Branch
Health Effects Division (7509C)

THROUGH: William L. Burnam, Branch Chief
Science Analysis Branch
Health Effects Division (7509C)

Background

A combined chronic toxicity and oncogenicity study in Crl: CD(SD)BR rats was conducted by Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England, for the Ziram Task Force, Sterling, Virginia, and completed September 27, 1994, (Study No. ZIR 9/942098; MRID No. 434042-01).

The study design allocated groups of 50 rats per sex per dose to dose levels of 0, 60, 180, or 540 ppm of Ziram for 105 weeks. An additional 20 rats per sex per dose were designated for interim sacrifice at week 53.

Survival Analyses

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of Ziram in

male rats. Female rats showed a significant decreasing trend in mortality with increasing doses of Ziram. See Tables 1 and 2 for mortality test results.

The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Tumor Analyses

Male rats had significant increasing trends at $p < 0.01$, and significant differences in the pair-wise comparisons of the 540 ppm dose group with the controls at $p < 0.05$, for mesenteric lymph node hemangiomas and for lymph node and spleen hemangiomas combined.

There were no compound-related tumors observed in female rats.

The statistical analyses of the male rats were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons. See Table 3 for tumor analysis results.

Table 1. Ziram - Crl: CD(SD)BR Rat Study

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks					Total
	1-26	27-52	53[i]	53-78	79-105[f]	
0	1/70	1/69	18/68	3/50	16/47	21/52 (40)
60	0/70	2/70	19/68	2/49	19/47	23/51 (45)
180	1/70	1/69	19/68	7/49	19/42	28/51 (55)
540	1/70	2/69	17/67	3/50	16/47	22/53 (42)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 53.

[f]Final sacrifice at week 105.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Ziram - Crl: CD(SD)BR Rat Study

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-26	27-52	53[i]	53-78	79-105[f]	Total
0	0/70	0/70	20/70	12/50	22/38	34/50 (68)**[n]
60	1/70	1/69	19/68	12/49	16/37	30/51 (59)
180	1/70	0/69	19/69	9/50	16/41	26/51 (51)*[n]
540	0/70	0/70	20/70	4/50	22/46	26/50 (52)**[n]

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 53.

[f]Final sacrifice at week 105.

[n]Negative trend or negative change from control.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 3. Ziram - Crl: CD(SD)BR Rat Study

Male Mesenteric Lymph Node and Spleen Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	60	180	540
Mesenteric Lymph Node Hemangiomas (%)	0/50 (0)	0/49 (0)	0/49 (0)	5[a]/49 (10)
p=	0.001**	1.000	1.000	0.027*
Spleen Hemangiomas (%)	0/50 (0)	0/49 (0)	0/49 (0)	1[b]/49 (2)
p=	0.249	1.000	1.000	0.495
Combined	0/50 (0)	0/49 (0)	0/49 (0)	6/49 (12)
p=	0.000**	1.000	1.000	0.012*

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a]First lymph node hemangioma observed at week 105, dose 540 ppm.

[b]First spleen hemangioma observed at week 102, dose 540 ppm.

Note: Interim sacrifice animals are not included in this analysis. There were no hemangiomas in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

References

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Thomas, D.G., N. Breslow, and J.J. Gart (1977) Trend and Homogeneity Analyses of Proportions and Life Table Data. Computers and Biomedical Research 10, 373-381.

Attachment 5

DATA EVALUATION REPORT

ZIRAM

Study Type: ONCOGENICITY FEEDING - MOUSE (83-2)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Health Sciences Research Division
Oak Ridge National Laboratory*
Oak Ridge, TN 37831
Task Order No. 94-43G

Primary Reviewer:
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Date: 9-19-95

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Signature: K.A. Davidson
Date: 9-18-95

Robert H. Ross, M.S., Group Leader

Signature: R.H. Ross for R.H. Ross
Date: 9-15-95

Quality Assurance:
Susan Chang, M.S.

Signature: S.S. Chang
Date: 9-18-95

Disclaimer

The final Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Oak Ridge National Laboratory personnel.

*Managed by Lockheed Martin Energy Systems, Inc., for the U.S. Department of Energy under Contract No. DE-AC05-84OR21400

94% of the control group. The mean weight gain in females was significantly decreased at 675 ppm compared to control values (80% of control). Dose-related decreases in mean absolute brain weights were seen in both sexes, but, although numerically greater in females, were statistically significant only in males at 225 and 675 ppm. The incidence of centrilobular hepatocyte enlargement was increased in all treated animals. The incidence reached maximums of about 50% in males and 38% in females at 75 and 225 ppm then dropped at the high dose to 39% in males and 14% in females. These effects seem to indicate an adaptive response at all doses since there was no effect on liver weight, no dose-related effect on the gradation of the pathology (minimal at all doses), and no necrosis seen even at the high dose. Significant increases in the incidences of urinary bladder epithelial cell hyperplasia were seen in males at 225 and 675 ppm (39 and 70%, respectively, in terminal animals compared to 18% in controls), and in females at 675 ppm (20% in terminal animals compared to 0 in controls). Urinary bladder epithelial hypertrophy was significantly increased in terminal females at 675 ppm (38% compared to 8% in controls).

The NOAEL is 75 ppm. The LOAEL is 225 ppm based on decreased absolute brain weights in both sexes and significantly increased incidence of urinary bladder epithelial hyperplasia and decreased body weight gain in males.

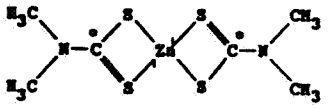
There were no treatment-related increases in tumor incidences.
This study is Acceptable/Guideline and satisfies the guideline requirements for a 83-2 oncogenicity feeding study in mice.

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS

1. Test material: Ziram

Description: white powder
Lot/Batch No.: 8331 AA
Purity: 98.7 % a.i.
Stability of compound: Test compound was assayed at 6-month intervals; no deterioration was detected when stored in the dark at ambient temperatures.
CAS No.: 137-30-4
Structure:



*Position of radiiolabel

2. Vehicle and/or positive control

of 900 and 2700 ppm Ziram. Effects on body weight were seen at 100, 300, 900, and 2700 ppm (MRID No.433737-01, Vol. 9, p. 1695).

2. Diet preparation and analysis

Diets were prepared weekly by mixing a weighed amount of test substance with a small amount of untreated basal diet in a Turbula mixer for 2 minutes to form a relatively concentrated pre-mix. This mixture was then diluted with an appropriate amount of food to achieve the desired concentrations and then mixed for 7 minutes in a double cone blender. The mixtures were divided into aliquots for daily use and stored at 4°C until immediately before feeding. The homogeneity of the Ziram-diet mixture at 25, 50, 70, and 5000 ppm was tested prior to the beginning of treatment. Samples of each dietary mixture were taken immediately after mixing (pre-dosing) and after storage for 6 days at 4°C and an additional 24 hours at room temperature (post-dosing) for weeks 1, 13, 26, 39, 52, 65, 73, and 78. Samples at week 73 were taken from only the 25 and 75 ppm groups because of a low analyzed concentration seen at week 65 for those groups. The pure compound was reassayed by the supplier at six month intervals.

Results –

a. Homogeneity analysis – The variation in the samples tested from the top to the bottom of the container ranged from -1.9% to +5.6% (coefficient of variation 2.8%) at 70 ppm, and from +4.6% to -3.0% (coefficient of variation 2.4%) at 5000 ppm (MRID 433737-01, p. 1646). Samples that were taken from 25 ppm and 50 ppm mixtures to reflect the low concentrations utilized in this study had coefficients of variation of 7.01 and 2.21, respectively (MRID 433737-01, Table 3, p. 1592).

b. Stability analysis – Ziram losses of 23% at 25 ppm, and 13% at 50 ppm occurred under the experimental conditions of this study (storage of the dietary mixture for 6 days at 4°C plus 24 hours at room temperature) (MRID 433737-01, Table 4, p. 1593). In agreement with the sponsor, it was decided to increase the concentration of the 25 ppm mixture to 29 ppm and the 75 ppm mixture to 83 ppm to compensate for the loss and approximate the mean target concentration.

c. Concentration analysis – The pre-dose samples from all dose levels were within +16 to -12% of the target dose with only 3 samples varying greater than ±10%. The post-dosing samples varied from +5 to -17% with the exception of one sample taken at the end of week 65 from the 25 ppm mixture which was 48% below the target dose. This result prompted a resampling of the 25 and 75 ppm mixtures at week 73. All but 5 of the post-dosing samples were within ±10% of the target concentration (MRID 433737-01, Table 1 pp.1584-1587).

3. Diet

Animals received food (ground SDS Rat and Mouse No.1 modified maintenance diet) and water *ad libitum.* Animals were given a fresh aliquot of food each day.

2. Males were more affected than females with significantly decreased weight gain of 23 and 44% less than the controls at 225 and 675 ppm, respectively. Males also showed a slight decrease of about 8% in overall weight gain at 75 ppm, but the decrease was not statistically significant. The body weights of all treated males were slightly lower than the control body weights from week 14 through week 80. The body weights of the high dose males ranged from about 79 to 83% of the control body weights. There was more variation in mean weight gain in females. Increases in mean weight gain of 16 and 21% were seen at 25 and 75 ppm, respectively. However, increased intragroup variation (standard deviations of 5.28 and 6.23, for 25 and 75 ppm, respectively) prevented the increases from reaching statistical significance. Decreases in mean weight gain of about 6 and 20% were seen in females at 225 and 675 ppm, respectively. A decrease in weight gain of about 22% was seen at 225 ppm compared to the mean weight at 75 ppm; however, compared to the controls, the decreased weight gain reached statistical significance only at the high dose in females. The body weights of the high dose female mice ranged from about 89 to 92% of the control body weights over the last 8 weeks of the study; whereas the body weights of the 75 ppm group were slightly but consistently higher ranging from about 105 to 109% of the control body weights.

TABLE 3. GROUP MEAN BODY WEIGHTS (g)

Treatment length (Weeks)	Males, Dose (ppm)					Females, Dose (ppm)				
	0	25[a]	75[b]	225	675	0	25	75	225	675
0	28	28	28	28	28	21	22	22	22	22
12	39	38	38	36	34	28 (46)[c]	28 (49)	28	27	27 (49)
24	44	42	43	39 (49)	36 (48)	31 (46)	32 (49)	32	29	29 (49)
36	47 (49)	45	44	41 (49)	38 (46)	33 (45)	34 (49)	34	32	31 (48)
52	48 (49)	46 (47)	46 (49)	42 (49)	39 (44)	34 (43)	36 (48)	36 (46)	33 (48)	32 (48)
68	48 (39)	46 (41)	46 (47)	42 (45)	39 (40)	35 (42)	37 (45)	38 (42)	34 (45)	33 (46)
80	48 (34)	47 (34)	46 (42)	43 (38)	39 (33)	36 (38)	38 (36)	39 (40)	35 (40)	33 (35)
Mean weight gain (g)[d]	19.7	19.0	18.2	15.1**	11.0**	14.1	16.4	17.0	13.2	11.3*

Data taken from MRID No. 433737-01, Volume 1, Table 2, p. 41-43.
[a]Prepared to contain 29 ppm.
[b]Prepared to contain 83 ppm.
[c](No. animals if less than 50)
[d]Weight gains taken from summary table on p. 28. Weight gain statistics were performed by study authors.
* $p<0.05$; ** $p<0.01$ significantly different from controls.

3. Food consumption and compound intake

TABLE 4. GROUP MEAN FOOD CONSUMPTION (g/ANIMAL/WEEK) IN MICE FED ZIRAM FOR 80 WEEKS

Week of Study	Treatment Group/Exposure Level (ppm)									
	Males					Females				
	0	25[a]	75[b]	225	675	0	25	75	225	675
1[c]	41	41	42	41	38	41	39	39	40	37**
12	40	37	36	33	30	42	37	33	30	29
24	43	40	38	37	33	44	39	37	33	30
36	39	39	36	31	32	40	37	34	32	30
52	41	42	39	33	32	43	38	35	31	29
68	44	39	35	32	32	43	38	36	32	30
80	46	38	34	33	32	43	36	36	33	30
Total food consumed (week 1-80)[c]	3267	3117 (95)	2886** (88)	2631** (81)	2517** (77)	3356	2923** (87)	2754** (82)	2499** (74)	2341** (70)
Food conversion ratios (week 1-28)[d]	64.9	71.8	69.1	83.7	102.6	116.3	92.8	88.6	101.7	107.6
Food efficiency (week 1-80)[e]	0.603	0.610	0.631	0.574	0.437	0.420	0.561	0.617	0.528	0.483

Data taken from MRID No. 433737-01, Table 3, pp. 44-46, and the summary table on p. 29.
[a]Prepared to contain 29 ppm.
[b]Prepared to contain 83 ppm.
[c]Taken from summary table on p. 29. Statistics on these values were performed by study authors.
[d]Food consumed (g) ÷ body weight gained (g). Taken from MRID No. 433737-01, Table 4, p. 47.
[e]Calculated by the reviewer from information in summary tables in MRID No. 433737-01, pp. 28-29.
* $p \leq 0.05$; ** $p \leq 0.01$ significantly different from controls.

b. Compound consumption (time-weighted average) – The overall compound consumption for weeks 1-80 calculated from the food consumption and body weights is given in Table 1. The study authors stressed that these values were calculated from the nominal dosages and not from the adjusted levels in the 25 and 75 ppm groups. The achieved group mean dosages were calculated each week throughout the experiment. The factor of 3 difference between groups was well maintained during the study.

c. Food efficiency – The food conversion ratios (food consumed ÷ body weight gained) calculated by the study authors for weeks 1-28 are inversely proportional to food efficiency values. These values generally indicate a slight decrease in food utilization in treated males up to week 80, and, although variable, the results indicate a slight increase in food utilization in females through this time period. The food efficiencies for the entire experiment calculated by the reviewer showed an increase in food efficiency of about 5% compared to the control animals in males at 75 ppm Ziram. However, decreases of 5 and 28% were seen at 225 and 675 ppm, respectively. An increase in food efficiency of about 47% was seen in females at 75 ppm Ziram. The food efficiency at the high dose

TABLE 5. DIFFERENTIAL LEUKOCYTE COUNT

Dose/ Treatment period	Percent of Total Count									
	Cell Type, Males					Cell Type, Females				
	Neut	Lymp	Eosin	Baso	Mono	Neut	Lymp	Eosin	Baso	Mono
Control 52 Week	30	69	1	0	0	22	75	1	0	1
675 ppm 52 Week	26	74	0*	0	0	27	72	1	0	0
Control Terminal	34	64	2	0	0	44	54	2	0	1
675 ppm Terminal	40	58	2	0	1	34*	64*	2	0	0

Data taken from MRID No. 433737-01, Table 6, p. 51.
* p≤.05

6. Urinalysis

Urinalysis is not required for an 83-2 oncogenesis study.

7. Sacrifice and pathology

Following 80 weeks of treatment, all surviving animals were killed by carbon dioxide asphyxiation, weighed and subjected to gross and microscopic necropsy procedures. All animals that died or were killed prematurely during the experiment were also subjected to gross and microscopic examination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed. The tissues, with the exception of the eyes, were preserved for examination in 10% formalin solution. The eyes were preserved in Davidson's fixative.

TABLE 6. ABSOLUTE AND RELATIVE ORGAN WEIGHTS (mg) IN MICE TREATED WITH ZIRAM FOR 80 WEEKS

Organ or Tissue	Treatment Group/Exposure Level (ppm)									
	Males					Females				
	0	25	75	225	675	0	25	75	225	675
Liver	2700 (589)[a]	2540 (560)	2500 (558)	2250 (541)	2020 (537)	1780 (525)	1750 (487)	1850 (500)	1720 (523)	1700 (553)
Kidneys	757 (165)	819 (181)	778 (174)	736 (180)	743 (199)**	511 (156)	471 (132)	485 (132)	475 (145)	460 (150)
Testes + Epidids.	336 (74.0)	336 (74.5)	326 (72.8)	341 (83.1)*	324 (87.8)**	---	---	---	---	---
Brain	479 (105)	483 (108)	473 (106)	468* (115)*	458** (125)**	505 (151)	497 (140)	488 (134)	471 (145)	459 (150)
Body Wt. (g)	46.2	45.7	45.2	41.6	37.5	34.1	36.5	37.4	32.9	31.1

Data taken from MRID No. 433737-01, Tables 7 and 8, p. 52-54.
[a](Relative weight)
* $p \leq 0.05$; ** $p \leq 0.01$ significantly different from controls.

b. Gross pathology – The incidences of selected gross lesions that changed with Ziram treatment are summarized in Table 7. Decreases in the amount of adipose tissue in decedents were seen at 675 ppm in 65% of the males and in 60% of the females compared to decedent controls (31% of males; 33% of females). However, the incidence of animals with decreased adipose tissue was not increased in animals killed at study termination. The incidences of roughened and white forestomach were increased in females at 675 ppm compared to controls (14.3% compared to 2.6% in controls for roughened stomach, 20.0% compared to 7.9% in controls for white stomach), but not in males at study termination. Irregular cortical scarring of the kidneys was seen in 42.4% of males at 675 ppm compared to 14.7% in controls at study termination. The incidence of brown discoloration of the kidneys was also slightly increased (9.1%) at 675 ppm compared to controls (0) in males. Lung petechiae were seen in 32.4% of control males and in 7.9% of females at study termination, but were not seen in any animals following 80 weeks of treatment at 675 ppm. Lung congestion was seen in all groups of animals except for high dose terminal males in a non dose-related fashion.

TABLE 8. NUMBER OF MICE FED ZIRAM IN THEIR DIET FOR 80 WEEKS WITH NON-NEOPLASTIC LESIONS

Affected Organ or Tissue/ Lesion	Treatment Group Exposure Level (ppm)									
	Males					Females				
	0 (34/16)[a]	25 (34/16)	75 (41/9)	225 (38/12)	675 (33/17)	0 (38/12)	25 (35/15)	75 (40/10)	225 (40/10)	675 (35/15)
Liver/ Centrilobular hepatocyte enlargement	1 (1)[b]	16*** (5)	20*** (1)	19*** (6)*	13*** (6)*	0 (0)	10*** (2)	15*** (4)	15[c]*** (1)	5* (8)*
Liver/ Centrilobular hepatocyte enlargement (Total)[c]	2	21**	21**	25**	19**	0	12**	19**	16[c]**	13**
Liver/ Centrilobular hepatocyte vacuolation	29 (2)	4*** (1)	1*** (0)	0*** (1)	2*** (0)	20 (1)	10* (1)	4*** (0)	5[c]*** (0)	0*** (0)
Liver/ Centrilobular hepatocyte enlargement and vacuolation	0 (0)	7** (0)	9** (0)	5* (0)	5* (1)	0 (0)	0 (0)	2 (0)	1[c] (0)	1 (0)
Liver/ Centrilobular hepatocyte enlargement and vacuolation (Total)[c]	0	7**	9**	5*	5*	0	0	2	1[c]	1
Liver/ Generalized enlargement	1 (0)	5 (4)	9* (3)*	13*** (2)	1 (5)*	0 (1)	4 (6)	7 (3)	5[c] (2)	5 (2)
Liver/ Generalized enlargement (Total)[c]	1	9**	12**	15**	6	1	10**	10**	7[c]*	7*
Urinary bladder/ Epithelial hyperplasia	6 (1)	5 (2)	5[d] (4)	15* (5)	23*** (8)	0[f] (0)	3 (2)	1[g] (0)	3[c] (2)	7[h]* (7)
Urinary bladder/ Epithelial hyperplasia (Total)[c]	7	7	9[d]	20**	31**	0[f]	5*	1[g]	5[c]*	14[h]**
Urinary bladder/ Epithelial hypertrophy	5 (2)	0 (2)	0[d] (1)	0 (1)	2 (1)	3[f] (3)	0 (0)	0[g] (2)	0[c] (0)	13[h]** (1)
Lungs/ Congestion	3 (5)	10*[i] (8)	7 (1)	7 (7)	3 (3)	8 (6)	9 (4)	15 (4)	9[c] (2)	5 (5)

Data taken from MRID No. 433737-01, Table 10, pp. 68-108.

[a](No. animals at termination of study/no. animals that died or were killed during the study)

[b]Incidence in animals at termination of study (Incidence in predecedents)

[c]Taken from MRID No. 433737-01, summary tables on p. 33; statistics performed by study authors.

[d]Only 37 animals were examined for this lesion at the termination of the study.

No significant differences were seen in the distribution of premature deaths among the controls and various dose groups. The highest survival rates (males, 84 and 76% at 75 and 225 ppm, respectively; females, 80% at 75 and 225 ppm) were seen in the middle dose groups in both sexes. Decreased weight gain in males was dose dependent and reached statistical significance at 225 and 675 ppm (77 and 56% of control weight gain, respectively). The body weight gain in females was much less affected and significant only at the high dose (80% of control weight gain). The mean body weight of females in the treated groups increased at 25 and 75 ppm and decreased at the higher doses, but never varied more than ±11% from the control body weights. However, the mean body weight of the 675 ppm dose group was 15.4% lower than the 75 ppm group. The overall food efficiency increased to about 105% and 147% of control levels in males and females, respectively at 75 ppm then decreased at 225 and 675 ppm. The food efficiency in females remained slightly above control levels, however in males it decreased to about 73% of control levels at the high dose. There seemed to be no correlation between food consumption and body weight in females.

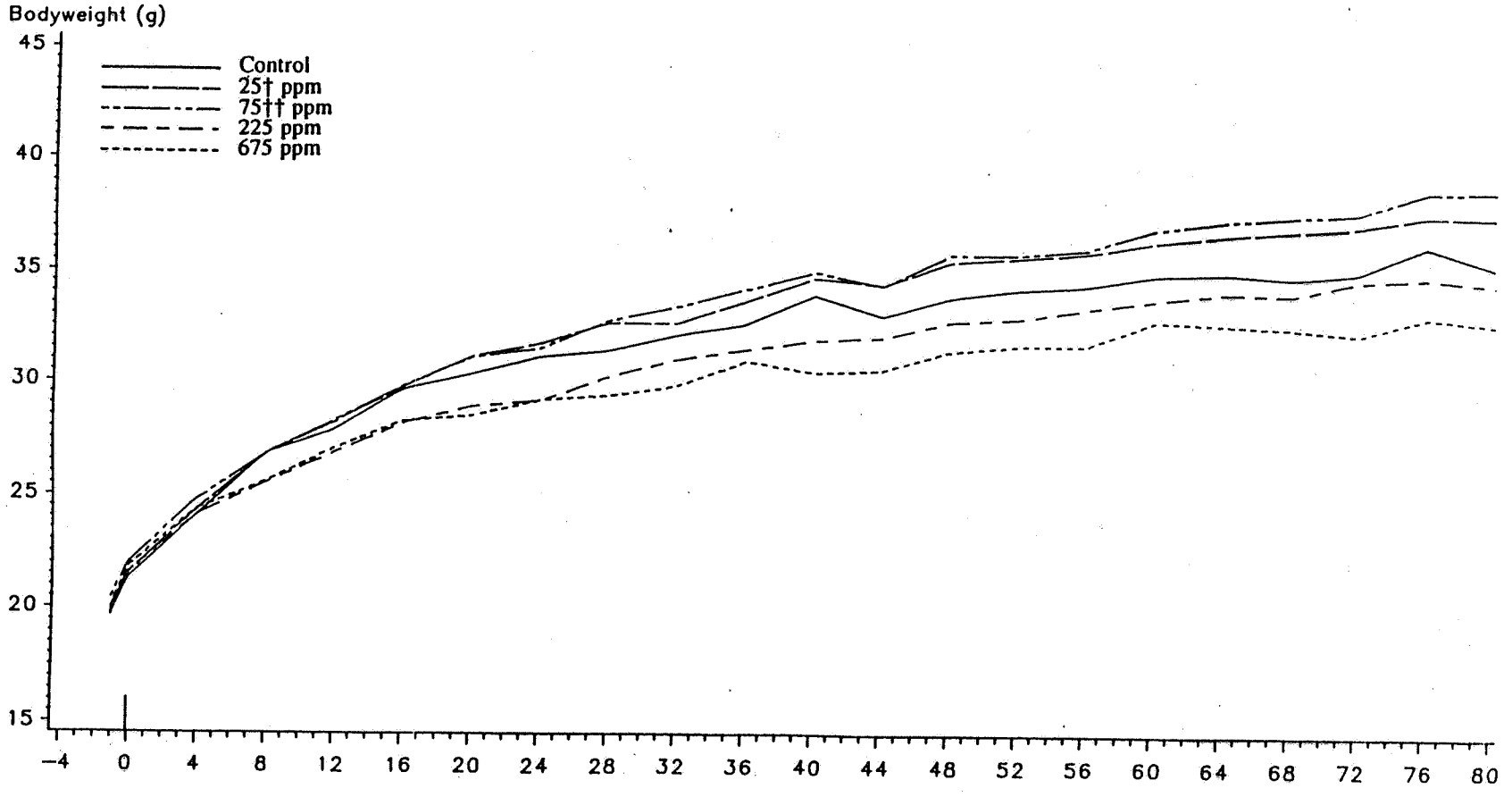
Significant changes in organ weights were seen in males including increases in the relative weights of kidneys (121% of control at 675 ppm), testes+epididymides (112 and 119% at 225 and 675 ppm, respectively), and brain (110 and 119% at 225 and 675 ppm, respectively). The absolute brain weights were slightly, but significantly decreased at 225 and 675 ppm compared to controls (98 and 96% of controls, respectively). The mean absolute brain weights of females were also decreased, but did not reach statistical significance (96.6, 93.3, and 90.9% of controls at 75, 225, and 675 ppm respectively). Covariant analysis of female brain weights correcting for terminal body weights also indicated slight, but significant, decreases in brain weight (96.2, 93.5, and 91.5% of control brain weight at 75, 225 and 675 ppm, respectively). Although the adjusted brain weights of terminal females receiving 75 ppm Ziram were slightly decreased, there were no increases in microscopic lesions found associated with the decreased brain weight. The significance of the adjusted brain weight at 75 ppm would seem more likely to reflect the varying body weight changes than the decreased brain weight. At higher doses the mean female body weights were less than the control level, and the mean absolute brain weight was decreased compared to controls. There were no significant additional organ weight changes in females. It would seem that the relative organ weight changes can largely be explained on the basis of decreased body weight seen especially in males at 225 and 675 ppm Ziram.

Gross pathological examination of tissues and organs found random lung congestion with a decrease in lung petechiae, especially in treated males; a slight decrease in adipose tissue in females; increases in roughened and white appearance of the stomach in females at the high dose; and increases in irregular cortical scarring ($p<0.05$) and brown coloration of the kidneys in males. Only the cortical scarring of the kidneys in high dose males reached statistical significance, and no microscopic lesions were found in the stomachs or kidneys of the test animals. Microscopic examination revealed dose-dependent urinary bladder epithelial cell hyperplasia in both sexes. This lesion was found in 62.0% of high dose males in the study compared with 14.0% in the controls, and in 28.0% of high dose females compared to 0 in the controls. An increase in the incidence of urinary bladder epithelial hypertrophy was seen in terminal females at the high dose (38% compared to 8% in controls), but was not seen in males or in decedents. Significantly increased incidences of centrilobular hepatocyte enlargement were

APPENDIX

FIGURE 4

Bodyweights - group mean values - females



† Prepared to contain 29 ppm (+15%)
†† Prepared to contain 83 ppm (+10%)

S6

Mortality will be analysed using logrank methods (5). Fisher's test (6) will be applied to all macroscopic and microscopic pathological findings. In addition for selected tumours, incidence rates will be analysed according to the IARC recommendations (7). In such cases the context of observation of the tumour will be determined by the pathologist. Trend tests will be used, based on nominal (i.e. target) dose levels.

Additional or alternative statistical methods will be used when considered appropriate.

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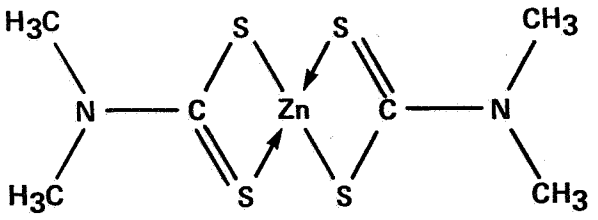
Attachment 6

CARCINOGENESIS BIOASSAY OF ZIRAM



ZIRAM

CAS NO. 137-30-4
$C_6H_{12}N_2S_4Zn$ Mol. Wt. 305.82

ABSTRACT

A carcinogenesis bioassay of ziram (89% pure, with 6.5% thiram), a fungicide and a rubber vulcanization accelerator, was conducted in F344/N rats and in B6C3F$_1$ mice. Groups of 50 rats of each sex received diets containing 300 or 600 ppm of commercial grade ziram for 103 weeks; groups of 49 or 50 mice of each sex received diets containing 600 or 1,200 ppm ziram; and groups of 50 rats and 50 mice of each sex served as untreated controls.

The average daily consumption of ziram by low- and high-dose rats, through the majority of the study, was about 11 and 22 mg/kg for males and 13 and 26 mg/kg for females. The average daily consumption of ziram by low- and high-dose mice, through the majority of the study, was 122 and 196 mg/kg for males and about 131 and 248 mg/kg for females.

Survival and feed consumption and mean body weights of rats of each sex were not adversely affected by ziram; rats of each sex possibly could have tolerated higher doses.

C-Cell carcinomas of the thyroid in male rats occurred with a statistically significant positive trend ($P<0.01$) and the incidence in the high-dose group was significantly higher ($P<0.05$) than that in the controls (control, 0/50, 0%; low dose, 2/49, 4%; high dose, 7/49, 14%) and higher than that previously observed in control male rats at the same laboratory (18/584, 3%; range 0% to 8%). The combined incidence of males with either C-cell adenoma or carcinoma also showed a statistically significant ($P<0.05$) positive trend (control, 4/50, 8%; low dose, 9/49, 18%; high dose, 12/49, 24%). There were no significant histopathologic changes noted in the follicular cells.

Survival of male and female mice was not adversely affected by ziram in feed; mean body weight gain by dosed male mice throughout the study and by high-dose female mice after week 80 was depressed by 15% to 20% relative to the controls. Average daily feed consumption by high-dose males and high-dose females was, respectively, 78% and 85% that of the controls. Mice probably could not have tolerated higher doses.

The incidence of alveolar/bronchiolar adenomas was significantly ($P<0.05$) increased in female mice (control, 2/50, 4%; low-dose, 5/49, 10%; high-dose, 10/50, 20%). The combined incidence of alveolar/bronchiolar adenomas or carcinomas in female mice showed a statistically significant ($P<0.05$) positive trend. The incidence in the high-dose group was significantly ($P<0.05$) higher than that in the controls (control, 4/50, 8%; low-dose, 6/49, 12%; high-dose, 11/50, 22%). Pulmonary adenomatous hyperplasia consistent with chronic Sendai virus infection (confirmed by serologic analyses performed on untreated animals from the same animal shipment and present in the same room) was observed in control and dosed male mice (control, 15/49, 31%; low-dose, 19/50, 38%; high-dose, 16/49, 33%) as well as in control and dosed female mice (control, 18/50, 36%; low-dose, 27/49, 55%; high-dose, 26/50, 52%). Six of the 26 high-dose females with the adenomatous hyperplasia had pulmonary tumors, whereas 4 of the 24 high-dose females without pulmonary adenomatous hyperplasia also had pulmonary tumors. Only 1 of 27 low-dose females with adenomatous hyperplasia had a pulmonary tumor.

There was a significant decrease in the incidence of mammary fibroadenomas in high-dose female rats (control, 16/50, 32%; low-dose, 17/50, 34%; high-dose, 8/50, 16%). Significant dose-related decreased incidences of liver carcinomas in male mice (control, 13/49, 27%; low-dose, 8/50, 16%; high-dose, 1/49, 2%) and of liver adenomas in female mice (control, 7/50, 14%; low-dose, 2/50, 4%; high-dose, 0/50, 0%) were observed.

Under the conditions of these studies, ziram was carcinogenic for male F344/N rats, causing increased incidences of C-cell carcinomas of the thyroid gland. Ziram was not carcinogenic for either female F344/N rats or for male $B6C3F_1$ mice. Increased incidences of alveolar/bronchiolar adenomas and of combined alveolar/bronchiolar adenomas or carcinomas occurred in female $B6C3F_1$ mice. However, the interpretation of this increase in lung tumors is complicated by an intercurrent Sendai virus infection.

TABLE 8. ANALYSIS OF PRIMARY TUMORS IN MALE RATS *(a)*

	Control	Low Dose	High Dose
Subcutaneous Tissue: Fibroma			
Tumor Rates			
Overall *(b)*	2/50(4%)	6/50(12%)	0/50(0%)
Adjusted *(c)*	6.1%	14.2%	0.0%
Terminal *(d)*	2/33(6%)	1/34(3%)	0/40(0%)
Statistical Tests *(e)*			
Life Table	P=0.222N	P=0.145	P=0.197N
Incidental Tumor Test	P=0.440N	P=0.099	P=0.197N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.253N	P=0.134	P=0.247N
Hematopoietic System: Undifferentiated Leukemia			
Tumor Rates			
Overall *(b)*	10/50(20%)	11/50(22%)	10/50(20%)
Adjusted *(c)*	25.3%	26.8%	22.5%
Terminal *(d)*	5/33(15%)	5/34(15%)	6/40(15%)
Statistical Tests *(e)*			
Life Table	P=0.408N	P=0.516	P=0.451N
Incidental Tumor Test	P=0.389	P=0.401	P=0.521
Cochran-Armitage Trend, Fisher Exact Tests	P=0.549	P=0.500	P=0.598
Hematopoietic System: Lymphoma or Leukemia			
Tumor Rates			
Overall *(b)*	10/50(20%)	11/50(22%)	11/50(22%)
Adjusted *(c)*	25.3%	26.8%	24.2%
Terminal *(d)*	5/33(15%)	5/34(15%)	6/40(15%)
Statistical Tests *(e)*			
Life Table	P=0.497N	P=0.516	P=0.541N
Incidental Tumor Test	P=0.275	P=0.401	P=0.386
Cochran-Armitage Trend, Fisher Exact Tests	P=0.452	P=0.500	P=0.500
Pituitary: Adenoma			
Tumor Rates			
Overall *(b)*	13/50(26%)	9/50(18%)	8/49(16%)
Adjusted *(c)*	32.5%	25.2%	19.3%
Terminal *(d)*	7/33(21%)	8/34(24%)	6/39(15%)
Statistical Tests *(e)*			
Life Table	P=0.078N	P=0.231N	P=0.102N
Incidental Tumor Test	P=0.185N	P=0.274N	P=0.273N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.141N	P=0.235N	P=0.176N
Pituitary: Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	15/50(30%)	11/50(22%)	10/49(20%)
Adjusted *(c)*	37.7%	30.9%	23.3%
Terminal *(d)*	9/33(27%)	10/34(29%)	7/39(18%)
Statistical Tests *(e)*			
Life Table	P=0.082N	P=0.238N	P=0.107N
Incidental Tumor Test	P=0.166N	P=0.281N	P=0.231N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.160N	P=0.247N	P=0.193N

TABLE 8. ANALYSIS OF PRIMARY TUMORS IN MALE RATS *(a)* (Continued)

	Control	Low Dose	High Dose
Adrenal: Pheochromocytoma			
Tumor Rates			
Overall *(b)*	7/50(14%)	6/50(12%)	7/50(14%)
Adjusted *(c)*	17.5%	15.9%	17.5%
Terminal *(d)*	3/33(9%)	4/34(12%)	7/40(18%)
Statistical Tests *(e)*			
Life Table	P=0.443N	P=0.493N	P=0.494N
Incidental Tumor Test	P=0.457	P=0.562N	P=0.504
Cochran-Armitage Trend, Fisher Exact Tests	P=0.558	P=0.500N	P=0.613
Thyroid: Follicular-Cell Carcinoma			
Tumor Rates			
Overall *(b)*	1/50(2%)	3/49(6%)	1/49(2%)
Adjusted *(c)*	2.6%	8.1%	2.6%
Terminal *(d)*	0/33(0%)	2/34(6%)	1/39(3%)
Statistical Tests *(e)*			
Life Table	P=0.563N	P=0.317	P=0.734N
Incidental Tumor Test	P=0.563	P=0.275	P=0.716
Cochran-Armitage Trend, Fisher Exact Tests	P=0.602	P=0.301	P=0.747
Thyroid: Follicular-Cell Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	1/50(2%)	5/49(10%)	1/49(2%)
Adjusted *(c)*	2.6%	13.9%	2.6%
Terminal *(d)*	0/33(0%)	4/34(12%)	1/39(3%)
Statistical Tests *(e)*			
Life Table	P=0.533N	P=0.113	P=0.734N
Incidental Tumor Test	P=0.575	P=0.094	P=0.716
Cochran-Armitage Trend, Fisher Exact Tests	P=0.584	P=0.098	P=0.747
Thyroid: C-Cell Adenoma			
Tumor Rates			
Overall *(b)*	4/50(8%)	7/49(14%)	5/49(10%)
Adjusted *(c)*	12.1%	18.2%	12.8%
Terminal *(d)*	4/33(12%)	4/34(12%)	5/39(13%)
Statistical Tests *(e)*			
Life Table	P=0.538	P=0.281	P=0.605
Incidental Tumor Test	P=0.456	P=0.243	P=0.605
Cochran-Armitage Trend, Fisher Exact Tests	P=0.422	P=0.251	P=0.487
Thyroid: C-Cell Carcinoma			
Tumor Rates			
Overall *(b)*	0/50(0%)	2/49(4%)	7/49(14%)
Adjusted *(c)*	0.0%	5.9%	17.9%
Terminal *(d)*	0/33(0%)	2/34(6%)	7/39(18%)
Statistical Tests *(e)*			
Life Table	P=0.006	P=0.245	P=0.016
Incidental Tumor Test	P=0.006	P=0.245	P=0.016
Cochran-Armitage Trend, Fisher Exact Tests	P=0.003	P=0.242	P=0.006

TABLE 8. ANALYSIS OF PRIMARY TUMORS IN MALE RATS *(a)* (Continued)

	Control	Low Dose	High Dose
Thyroid: C-Cell Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	4/50(8%)	9/49(18%)	12/49(24%)
Adjusted *(c)*	12.1%	23.7%	30.8%
Terminal *(d)*	4/33(12%)	6/34(18%)	12/39(31%)
Statistical Tests *(e)*			
Life Table	P=0.048	P=0.132	P=0.055
Incidental Tumor Test	P=0.032	P=0.109	P=0.055
Cochran-Armitage Trend, Fisher Exact Tests	P=0.020	P=0.109	P=0.024
Pancreatic Islets: Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	2/50(4%)	4/50(8%)	3/50(6%)
Adjusted *(c)*	6.1%	10.7%	7.0%
Terminal *(d)*	2/33(6%)	2/34(6%)	1/40(3%)
Statistical Tests *(e)*			
Life Table	P=0.499	P=0.350	P=0.577
Incidental Tumor Test	P=0.343	P=0.316	P=0.445
Cochran-Armitage Trend, Fisher Exact Tests	P=0.417	P=0.339	P=0.500
Preputial Gland: Adenoma			
Tumor Rates			
Overall *(b)*	3/50(6%)	5/50(10%)	2/50(4%)
Adjusted *(c)*	7.9%	14.7%	5.0%
Terminal *(d)*	1/33(3%)	5/34(15%)	2/40(5%)
Statistical Tests *(e)*			
Life Table	P=0.337N	P=0.373	P=0.437N
Incidental Tumor Test	P=0.399N	P=0.342	P=0.555N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.421N	P=0.357	P=0.500N
Preputial Gland: Carcinoma			
Tumor Rates			
Overall *(b)*	4/50(8%)	3/50(6%)	4/50(8%)
Adjusted *(c)*	10.8%	6.9%	9.1%
Terminal *(d)*	1/33(3%)	0/34(0%)	2/40(5%)
Statistical Tests *(e)*			
Life Table	P=0.519N	P=0.494N	P=0.573N
Incidental Tumor Test	P=0.407	P=0.498N	P=0.413
Cochran-Armitage Trend, Fisher Exact Tests	P=0.576	P=0.500N	P=0.643
Preputial Gland: Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	7/50(14%)	8/50(16%)	6/50(12%)
Adjusted *(c)*	17.9%	20.6%	13.9%
Terminal *(d)*	2/33(6%)	5/34(15%)	4/40(10%)
Statistical Tests *(e)*			
Life Table	P=0.517	P=0.407N	P=0.407N
Incidental Tumor Test	P=0.551N	P=0.489	P=0.518
Cochran-Armitage Trend, Fisher Exact Tests	P=0.443N	P=0.500	P=0.500N

TABLE 8. ANALYSIS OF PRIMARY TUMORS IN MALE RATS *(a)* (Continued)

	Control	Low Dose	High Dose
Testis: Interstitial-Cell Tumor			
Tumor Rates			
Overall *(b)*	41/50(82%)	42/50(84%)	45/50(90%)
Adjusted *(c)*	93.0%	93.3%	93.7%
Terminal *(d)*	30/33(91%)	31/34(91%)	37/40(93%)
Statistical Tests *(e)*			
Life Table	P=0.317N	P=0.560	P=0.351N
Incidental Tumor Test	P=0.119	P=0.338	P=0.191
Cochran-Armitage Trend, Fisher Exact Tests	P=0.162	P=0.500	P=0.194

(a) Dosed groups received doses of 300 or 600 ppm of ziram in the diet.
(b) Number of tumor bearing animals/number of animals examined at the site.
(c) Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.
(d) Observed tumor incidence at terminal kill.
(d) Beneath the control incidence are the P-values associated with the trend test. Beneath the dosed group incidence are the P-values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying before the terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as non-fatal. The Cochran-Armitage and Fisher's exact tests compare directly the overall incidence rates. A negative trend is indicated by (N).

Pathology and Statistical Analyses of Results

Histopathologic findings on neoplasms in rats are summarized in Appendix A, Tables A1 and A2; Tables A3 and A4 give the survival and tumor status for each individual animal in the male and female rat studies, respectively. Findings on nonneoplastic lesions are summarized in Appendix C, Tables C1 and C2. Tables 8 and 9 contain the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups.

Thyroid: C-cell carcinomas occurred at a significantly increased incidence ($P<0.05$) in high-dose male rats, and with a significant ($P<0.01$) dose-related trend (control 0/50; low-dose, 2/49, 4%; high-dose, 7/49, 14%). The dose-related trend was significant ($P<0.05$) for male rats with C-cell adenomas or carcinomas (control 4/50, 8%; low-dose, 9/49, 18%; high-dose 12/49; 24%). Neither C-cell adenomas nor C-cell carcinomas were significantly increased in dosed female rats. C-cell hyperplasia of the thyroid gland was observed in male rats (control, 7/50, 14%; low-dose, 12/49, 24%; high-dose, 11/49, 22%) and in female rats (control, 16/50, 32%; low-dose, 11/50, 22%; high-dose, 19/50, 38%). Thyroglossal duct cysts occurred in male rats (control, 0/50; low-dose 3/49, 6%; high-dose, 1/49, 2%) and in female rats (control, 0/50; low-dose, 7/50, 14%; high-dose, 5/50, 10%). Follicular-cell adenomas or carcinomas occurred at all incidences in all groups of male and female rats (Tables A1 and A2).

Mammary Gland: Fibroadenomas were observed in decreased incidence in the mammary gland of high-dose female rats ($P<0.05$), even though more high-dose than control females lived to the end of the study. There was evidence of a dose-related decrease in the incidence of females with adenocarcinomas ($P=0.040$, life table trend test).

Eye: Retinopathy was observed at increased incidences in high-dose males and in dosed females (control males, 32/50, 64%; low-dose males, 7/50, 14%; high-dose males, 45/50, 90%; control females, 9/50, 18%; low-dose females, 48/50, 96%; high-dose females, 30/50, 60%).

Attachment 7

TABLE 17. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE *(a)*

	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma			
Tumor Rates			
Overall *(b)*	2/50(4%)	5/49(10%)	10/50(20%)
Adjusted *(c)*	5.9%	12.0%	24.4%
Terminal *(d)*	1/32(3%)	4/40(10%)	9/40(23%)
Statistical Tests *(e)*			
Life Table	P=0.022	P=0.311	P=0.041
Incidental Tumor Test	P=0.012	P=0.248	P=0.024
Cochran-Armitage Trend, Fisher Exact Tests	P=0.009	P=0.210	P=0.014
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	4/50(8%)	6/49(12%)	11/50(22%)
Adjusted *(c)*	10.1%	14.2%	26.8%
Terminal *(d)*	1/32(3%)	4/40(10%)	10/40(25%)
Statistical Tests *(e)*			
Life Table	P=0.071	P=0.486	P=0.108
Incidental Tumor Test	P=0.013	P=0.240	P=0.023
Cochran-Armitage Trend, Fisher Exact Tests	P=0.031	P=0.357	P=0.045
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type			
Tumor Rates			
Overall *(b)*	1/50(2%)	1/50(2%)	7/50(14%)
Adjusted *(c)*	3.1%	2.3%	16.9%
Terminal *(d)*	1/32(3%)	0/40(0%)	6/40(15%)
Statistical Tests *(e)*			
Life Table	P=0.019	P=0.713N	P=0.064
Incidental Tumor Test	P=0.011	P=0.755	P=0.049
Cochran-Armitage Trend, Fisher Exact Tests	P=0.011	P=0.753	P=0.030
Hematopoietic System: Malignant Lymphoma, Histiocytic Type			
Tumor Rates			
Overall *(b)*	0/50(0%)	4/50(8%)	2/50(4%)
Adjusted *(c)*	0.0%	10.0%	4.4%
Terminal *(d)*	0/32(0%)	4/40(10%)	0/40(0%)
Statistical Tests *(e)*			
Life Table	P=0.284	P=0.095	P=0.275
Incidental Tumor Test	P=0.180	P=0.095	P=0.073
Cochran-Armitage Trend, Fisher Exact Tests	P=0.222	P=0.059	P=0.247
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Tumor Rates			
Overall *(b)*	3/50(6%)	1/50(2%)	2/50(4%)
Adjusted *(c)*	8.4%	2.4%	5.0%
Terminal *(d)*	2/32(6%)	0/40(0%)	2/40(5%)
Statistical Tests *(e)*			
Life Table	P=0.328N	P=0.247N	P=0.416N
Incidental Tumor Test	P=0.447N	P=0.318N	P=0.529N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.400N	P=0.309N	P=0.500N

TABLE 17. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE *(a)* (Continued)

	Control	Low Dose	High Dose
Hematopoietic System: All Malignant Lymphoma			
Tumor Rates			
Overall *(b)*	6/50(12%)	6/50(12%)	12/50(24%)
Adjusted *(c)*	17.0%	14.2%	27.6%
Terminal *(d)*	4/32(13%)	4/40(10%)	9/40(23%)
Statistical Tests *(e)*			
Life Table	P=0.146	P=0.476N	P=0.212
Incidental Tumor Test	P=0.051	P=0.583N	P=0.073
Cochran-Armitage Trend, Fisher Exact Tests	P=0.067	P=0.620	P=0.096
Hematopoietic System: Lymphocytic Leukemia			
Tumor Rates			
Overall *(b)*	5/50(10%)	1/50(2%)	2/50(4%)
Adjusted *(c)*	11.3%	2.1%	5.0%
Terminal *(d)*	0/32(0%)	0/40(0%)	2/40(5%)
Statistical Tests *(e)*			
Life Table	P=0.110N	P=0.085N	P=0.181N
Incidental Tumor Test	P=0.591N	P=0.409N	P=0.657N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.133N	P=0.103N	P=0.218N
Hematopoietic System: Lymphoma or Leukemia			
Tumor Rates			
Overall *(b)*	11/50(22%)	7/50(14%)	14/50(28%)
Adjusted *(c)*	26.4%	16.0%	32.3%
Terminal *(d)*	4/32(13%)	4/40(10%)	11/40(28%)
Statistical Tests *(e)*			
Life Table	P=0.443	P=0.136N	P=0.520
Incidental Tumor Test	P=0.064	P=0.416N	P=0.093
Cochran-Armitage Trend, Fisher Exact Tests	P=0.271	P=0.218N	P=0.322
Liver: Adenoma			
Tumor Rates			
Overall *(b)*	7/50(14%)	2/50(4%)	0/50(0%)
Adjusted *(c)*	21.1%	5.0%	0.0%
Terminal *(d)*	6/32(19%)	2/40(5%)	0/40(0%)
Statistical Tests *(e)*			
Life Table	P=0.001N	P=0.041N	P=0.004N
Incidental Tumor Test	P=0.002N	P=0.048N	P=0.006N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.003N	P=0.080N	P=0.007N
Liver: Adenoma or Carcinoma			
Tumor Rates			
Overall *(b)*	9/50(18%)	4/50(8%)	1/50(2%)
Adjusted *(c)*	26.1%	10.0%	2.5%
Terminal *(d)*	7/32(22%)	4/40(10%)	1/40(3%)
Statistical Tests *(e)*			
Life Table	P=0.002N	P=0.055N	P=0.004N
Incidental Tumor Test	P=0.003N	P=0.070N	P=0.006N
Cochran-Armitage Trend, Fisher Exact Tests	P=0.005N	P=0.117N	P=0.008N

Ziram

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TABLE 17. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE *(a)* (Continued)

(a) Dosed groups received doses of 600 or 1,200 ppm of ziram in the diet.
(b) Number of tumor bearing animals/number of animals examined at the site.
(c) Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.
(d) Observed tumor incidence at terminal kill.
(e) Beneath the control incidence are the P-values associated with the trend test. Beneath the dosed group incidence are the P-values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying before the terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as non-fatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend is indicated by (N).

Attachment 8

DATA EVALUATION REPORT

ZIRAM

Study Type: CHRONIC FEEDING -DOG (83-1b)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Health Sciences Research Division
Oak Ridge National Laboratory*
Oak Ridge, TN 37831
Task Order No. 94-43 E, F

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Signature: Susan Chang
Date: 11-1-95

Disclaimer

The final Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Oak Ridge National Laboratory personnel.

*Managed by Lockheed Martin Energy Systems for the U.S. Department of Energy under Contract No. DE-AC05-84OR21400

There was a treatment-related convulsive episode at week 11 for a female in the 700/500 ppm dose group that required the animal to be euthanitized. In addition to the convulsive episode, the findings for the 700/500 ppm and 185 ppm dose groups include: 1) decreased body weight gain for females over the treatment period, 2) changes in clinical chemistry parameters-decreases in albumin (males and females) and total protein levels (females) and increases in SGPT (males) and alkaline phosphatase (males), and 3) histologic findings for livers (aggregates of Kupffer cells and macrophages and increased infiltration of inflammatory cells in males and females) and spleens (pigmented macrophages; males). **The NOAEL is 50 ppm based on the lack of significant toxicological effects. The LOAEL is 185 ppm based on decreased body weight gain in females, and increased liver pathology accompanied by corresponding clinical chemistry changes and the occurrence of pigmented macrophages in the spleen in males.**

Classification: This study is classified as **acceptable**. The study satisfies most of the guideline requirements for a chronic feeding study in beagle dogs (§83-1).

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS

1. Test material: Ziram (technical)

Description: white powder
Lot/Batch No.: 8331 AA
Purity: 98.5% ai.
Stability of compound: active ingredient content stable for 2 years
CAS No.: 137-30-4
Structure:

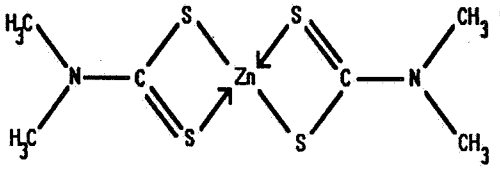

TABLE 1. STUDY DESIGN

Dose Group	Conc. in Diet (ppm)	Dose (mg/kg/day)		No. of Animals	
		Male	Female	Male	Female
1 Control	0	0.0	0.0	4	4
2 Low (LDT)	50	1.6	1.9	4	4
3 Mid (MDT)	185	6.6	6.7	4	4
4 High (HDT)	700/500	17.4	20.6	4	4/3[a]

Data taken from Table 4, pp. 63-64, MRID No. 428239-01.
[a]One female was sacrificed during week 11, after a convulsive episode. A second female was removed due to suspicion of polyarteritis during week 6, and a replacement female entered into the study at week 8.

2. Diet preparation and analysis

Diet was prepared weekly by mixing appropriate amounts of Ziram with ground standard dry diet (Diet A from Special Diets Services Ltd.) and was stored in daily aliquots, protected from light, at -20°C. Daily portions were fed and then discarded after 8 hours. Concentrated premixes were prepared by grinding appropriate amounts of Ziram and untreated basal diet in a turbula mixer for at least 2 minutes. The premixes were diluted to the appropriate concentrations by addition of basal diet and blending in a Gardner double-cone blender for at least 7 minutes. Diets for the 50 ppm group were prepared at 57.5 ppm in order to compensate for losses occurring during preparation (as determined by pre-study analyses). There were no significant losses for the diets prepared for the 185 or 700/500 ppm groups. Homogeneity and stability were tested at 5000, 100 and 50 ppm at room temperature. During the study, samples of treated food were analyzed at weeks 1, 13, 26, 39, and 52 for stability and concentration.

Results -

a. Homogeneity analysis - Diet was prepared with Ziram at concentrations of 50, 100, and 5000 ppm. Two samples from the top, middle, and bottom regions of each preparation were analyzed and were essentially homogeneous with respect to Ziram concentration.

b. Stability analysis - The stability of Ziram in diet preparations was assessed during four trials (Addendum 1, p. 287, MRID No. 428239-01). In the first two trials, stability at room temperature was assessed at the 100 and 5000 ppm inclusion levels. At 5000 ppm, Ziram mixed with diet was stable for 14 days (recovery of approximately 98%). However, at the 100 ppm inclusion level, losses occurred during storage at room temperature: ~16% after 24 hours, ~30% after 8 or 14 days. These losses were considered significant by the study authors. In trials three and

Results – One female (#368, 700 ppm dose group) was euthanitized on day 7 of week 11, after a suspected convulsion thought to be related to Ziram treatment (clinical signs: collapsed and salivating with trembling, champing of the jaws and jerking limbs). Dosage was decreased for all animals in the 700 ppm group to 500 ppm at day 3 of week 12. One other female (No. 372) in the 700 ppm dose group was sacrificed during week 6 due to a suspicion of polyarteritis. This suspicion was not confirmed upon pathological examination although a mild synovitis was discovered that likely contributed somewhat to the clinical signs. This animal was replaced by another female (No. 112) at week 8.

2. Body weight

Animals were weighed, before feeding, once each week for the 4 weeks prior to and for the 52 weeks of the treatment period.

Results – For males, there was no effect of Ziram treatment on weight gain (Table 2). For females treated with Ziram, there was a dose-dependent reduction in mean group weight gain and mean group body weights relative to controls (Table 2). From 0 to 52 weeks, mean weight gain for females treated with Ziram at 185 and 700/500 ppm was statistically significantly different from controls ($p<0.05$ and $p<0.01$, respectively). During the first 39 weeks of treatment, control and Ziram-treated female dogs gained weight at similar rates. From 39-52 weeks, mean weight gain for female dogs treated with Ziram at 185 and 700/500 ppm was statistically significantly ($p<0.05$) less than controls. At 52 weeks, mean group body weights for females were reduced, dose-dependently, compared to controls.

TABLE 3. GROUP MEAN FOOD CONSUMPTION, FOOD EFFICIENCY, AND COMPOUND INTAKE

Parameter	Exposure Level (ppm)							
	Males				Females			
	0	50	185	700/ 500	0	50	185	700/ 500
Food Consumption[a] (g/dog/day)	400	400	400	399.5	398.5	400	392.2	384.1
Total Food Consumption[a] (kg/dog)	145.6	145.6	145.6	145.4	145.1	145.6	142.8	139.8
Average Compound Intake (mg/kg/day)	—	1.6	6.6	17.4[b]	—	1.9	6.7	20.6[b]
Food Efficiency[a]	0.96	1.37	0.62	1.51	1.1	0.82	0.35	0.21

Data for calculation of food consumption taken from Table 3 (pp. 61-62), data for bodyweight gain taken from Table 2, pp. 59-60, data for average compound intake taken from Table 4 (pp.63-64), MRID No. 428239-01.
[a] Calculated by reviewer.
[b] Data reflect the initial dose of Ziram at 700 ppm, and the reduction to 500 ppm during week 12.

compound consumption (Table 3). Achieved intakes reflect the consumption of Ziram at the 700 ppm level and the reduction to 500 ppm at day 3 of week 12.

c. Food efficiency – Food efficiency was not calculated by the study authors. Using the data from Appendix 7 (Body weights, pp.161-168) and Appendix 8 (Food Consumption, pp.169-176), mean group food efficiency [(kg of weight gained per kg food consumed)x100] was calculated by the reviewer (Table 3). For males, food efficiency was quite variable, but the variations were not dose-related. For females, food efficiency decreased with increasing dose of Ziram.

4. Ophthalmoscopic examination

Prior to commencement of treatment, and at weeks 13, 26, and 52, the eyes of all animals were examined by means of a Keeler indirect ophthalmoscope. Pupils were dilated using Tropicamide ophthalmic solution ("Mydricyl," Alcon Laboratories, Inc.)

Results – No results attributable to treatment with Ziram. All findings considered typical of the age and strain of animals. One dog (male 361) developed a corneal ulcer with keratitis and associated conjunctivitis in week 32 due to a sawdust particle lodging in the eye. Surgery was performed under anesthesia and by week 37 no further treatment was necessary.

5. Blood was collected for hematology and clinical analysis from all animals after an overnight fast. Samples of blood were withdrawn from the jugular or cephalic vein at

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TABLE 4. HEMATOLOGY, WEEKS 13-52, MEANS OF SELECTED PARAMETERS

Males Week	Dose	RBC	MCHC	MCV	Total WBC	Lymph.	Mono.	Plts	APTT
13	0	5.9	29.9	79	9.5	2.61	0.09	385	12.5
	50	5.9	29.4	81	13.6	4.12	0.00	366	12.5
	185	5.5	29.9	78	11.3	3.51	0.16	392	13.4
	500	5.4	30.0	83**	13.5*	4.56*	0.37	374	14.3*
26	0	6.2	29.6	76	8.0	2.15	0.09	403	11.5
	50	6.6	29.1	76	8.0	2.62	0.06	440	12.2
	185	6.3	29.1	78	10.3	3.37	0.23	460	12.4
	500	5.9	28.8	80**	8.4	2.77	0.19	365	12.4
52	0	6.1	31.0	83	7.9	2.15	0.21	421	11.8
	50	6.7	30.7	82	9.6	2.36	0.30	394	11.9
	185	5.8	30.5	81	9.2	2.56	0.22	442	12.5
	500	5.9	29.7**	87*	10.0	3.47*	0.23	483	12.8
Females		**RBC**	**MCHC**	**MCV**	**Total WBC**	**Lymph.**	**Mono.**	**Plts**	**APTT**
13	0	6.4	29.4	80	9.8	3.02	0.27	328	13.0
	50	6.3	29.6	78	12.1	4.29	0.10	357	13.1
	185	5.9	29.7	80	16.3	5.24	0.11	444*	14.1
	500	5.8	28.3	85	12.1	4.35	0.27	480*	15.2**
26	0	6.7	29.2	77	8.2	2.45	0.11	365	12.7
	50	6.7	29.3	77	8.3	2.45	0.17	450	13.1
	185	5.8*	29.3	78	8.2	2.08	0.04	392	13.1
	500	6.0*	29.2	81*	10.3	2.90	0.34*	492*	15.8
52	0	6.7	30.3	83	10.4	2.72	0.35	446	13.4
	50	6.4	30.0	83	11.3	2.58	0.30	532	13.3
	185	6.5	30.3	84	11.7	3.01	0.25	501	13.7
	500	6.4	29.3	88	11.1	4.25	0.58	436	14.3

Data adapted from Table 7(pp.72-74), MRID No. 428239-01. Measurements: RBC ($x10^6/mm^3$), MCHC (%), MCV (fL), WBC (Total, L, and M: $x10^3/mm^3$), Plts ($x10^3/mm^3$), APTT (seconds).
*$p<0.05$, **$p<0.01$, Williams' test

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elevations, there were changes in other parameters that are likely of toxicological importance. For males, SGPT (GPT) and ALK (AP) levels were

TABLE 5. MEANS OF SELECTED CLINICAL CHEMISTRY PARAMETERS FOR WEEKS 13-52

Males Week	Dose	Prot.	ALB	Glob.	GGT	OCT	AP	GPT	CPK	Phosph.	Chol.	Bili.
13	0.00	5.5	2.7	2.8	2	4.1	126	23	98	3.4	140	0.1
	50	5.5	2.7	2.8	2	5.1	107	20	100	3.8	143	0.2
	185	5.6	2.7	2.9	2	2.7	133	21	115	3.6	150	0.1
	500	5.6	2.6	3.0	1**	2.6	161	21	102	4.0	205**	0.1
26	0.00	5.5	2.7	2.8	3	4.2	104	27	67	2.8	141	0.2
	50	5.5	2.8	2.8	2	5.2	84	33	75	3.7**	146	0.2
	185	5.5	2.7	2.8	2	6.0	143	70	82	3.2**	144	0.2
	500	5.4	2.4*	3.0	2*	6.1	148	86	101	3.7**	211**	0.2
52	0.00	5.7	3.0	2.8	3	2.9	99	29	55	2.6	142	0.1
	50	5.7	3.0	2.7	<2	3.3	81	23	62	3.0	156	0.1
	185	5.6	2.9	2.7	2	4.9	175	69	104*	2.9	140	0.1
	500	5.6	2.6*	2.9	<2	4.1	160	51	75*	3.0	214**	0.1
Females		**Prot.**	**ALB**	**Glob.**	**GGT**	**OCT**	**AP**	**GPT**	**CPK**	**Phosph.**	**Chol.**	**Bili.**
13	0.00	5.8	3.0	2.8	2	4.2	135	22	155	3.1	141	0.2
	50	5.4	2.7	2.6	1	4.8	113	21	135	3.3	125	0.2
	185	5.4	2.8	2.6	1	2.7	139	19	75	3.5	146	0.2
	500	5.2	2.6*	2.7	<1	3.4	175	21	115	3.7	162	0.1
26	0.00	5.7	2.9	2.8	2	4.2	120	25	104	2.6	152	0.2
	50	5.2	2.6	2.6	3	4.1	96	20	74	3.1	126	0.2
	185	5.4	2.6	2.8	2	5.8	125	18	67	2.9	171	0.2
	500	5.2*	2.4*	2.7	2	3.8	144	19	91	3.4*	174	0.2
52	0.00	6.0	3.2	2.8	<2	2.2	119	20	53	2.6	189	0.1
	50	5.4*	2.8	2.6*	<2	2.9	100	20	69	2.7	125	0.2
	185	5.2*	2.7*	2.5*	3	5.3*	109	21	64	3.3*	153	0.2*
	500	5.2*	2.7*	2.5*	1	4.8*	151	19	57	3.2*	183	0.2**

Data adapted from Table 8 (pp. 75-78), MRID No. 428239-01. Units: Total Protein (Prot.), ALB, and Globulin fraction (Glob.) were measured in g/dL, GGT, OTC,AP, GPT and CPK were measured in (mU/mL), P (mEq/L), and Cholesterol levels (Chol.) were measured in mg/dL. *p<0.05, Williams' test. **p<0.01, Williams' test.

Parathyroids and thyroids were weighed together as were testes and epididymides. Certain tissues were not taken from female dogs 368 and 372 (700/500 ppm group, sacrificed in week 11 and 6, respectively): median nerves, sciatic nerves (proximal), semilunar ganglia, spinal cord (C6-67, T5-T8, ventral roots and dorsal root ganglia, and tibial nerves. All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	Digestive system
X	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
X	Gall Bladder*
XX	Pancreas*
	Respiratory
X	Trachea*
XX	Lung*
	Nose
	Pharynx
	Larynx

X	Cardiovasc./Hemat.
X	Aorta*
XX	Heart*
X	Bone marrow*
X	Lymph nodes*
XX	Spleen
XX	Thymus*
	Urogenital
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
XX	Epididymides
XX	Prostate
	Seminal vesicle
XX	Ovaries*+
XX	Uterus*

X	Neurologic
XX	Brain*+
X	Periph. nerve*
X	Spinal cord (3 levels)*
XX	Pituitary*
X	eye (optic n.)*
	Glandular
XX	Adrenal gland*
	Lacrimal gland
X	Mammary gland*
XX	Parathyroids*++
XX	Thyroids*++
	Other
X	Bone*
X	Skeletal muscle*
X	Skin*
	All gross lesions and masses*

* Required for subchronic and chronic studies.
+ Organ weight required in subchronic and chronic studies.
++Organ weight required for non-rodent studies.

Results –

a. Organ weight – The mean group liver weight for males treated with Ziram at 700/500 ppm was significantly increased relative to controls ($p < 0.05$, Table 6), due mainly to increased liver weights for males 365 and 367. Liver weights for these dogs equaled or exceeded the expected upper limit of liver weight of 4% of

TABLE 7. DEGREE OF MICROSCOPIC PATHOLOGY-INDIVIDUAL ANIMALS

Pathology	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	50	185	700/ 500	0	50	185	700/ 500
LIVER (1) Foci of degenerate hepat. Incidence/Total # of animals	1/4	2/4	2/4	3/4	3/4	0/4	3/4	2/3
Individual animals: Moderate	---	---	---	371	---	---	358	112, 366
Minimal	---	349	359, 363	369	342,344	---	360, 362	---
Trace	345	353	---	367	346	---	---	---
(2) Infiltration of inflammatory cells: (a) around central veins Incidence/Total No. of animals	0/4	0/4	0/4	1/4	0/4	1/4	2/4	1/3
Individual animals: Minimal	---	---	---	367	---	354	358, 360	366
(b) around central veins and branches of the hepatic vein Incidence/Total No. of animals	0/4	0/4	2/4	3/4	0/4	0/4	0/4	1/3
Individual animals: Moderate	---	---	---	371	---	---	---	---
Minimal	---	---	357, 359	365, 369	---	---	---	370
(c) around portal areas Incidence/Total No. of animals	1/4	0/4	1/4	3/4	0/4	0/4	0/4	1/3
Individual animals: Minimal	343 (foci)	---	359	367, 369, 371	---	---	---	370
(3) Single cell necrosis Incidence/Total No. of animals	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/3
Individual animals: Minimal	---	---	---	371	---	---	---	---
(4) Increase in centrilobular fibrocytes Incidence/Total No. of animals	0/4	0/4	1/4	3/4	0/4	0/4	0/4	0/3
Individual animals (Not graded)	---	---	359	367, 369, 371	---	---	---	---
(5) Aggregates of pigmented Kupffer cells and macrophages Incidence/Total No. of animals	0/4	1/4	2/4	4*/4	0/4	1/4	4*/4	2/3

central veins and hepatic veins (2♂, 2♀), and moderate (2♂, 2♀) to minimal (2♀) amounts of aggregates of pigmented Kupffer cells and macrophages. The incidence of aggregates of pigmented Kupffer cells and macrophages for females was statistically significantly increased compared to controls ($p<0.05$). In addition, female 358 showed a focus of hepatic necrosis. In the spleen, there were moderate (1♂) to minimal (2♂, 2♀) amounts of pigmented macrophages.

50 ppm dose – There were few significant pathologic findings for this dose group (Table 7). For liver, there was minimal (1♂) or trace (1♂) amounts, or no detectable (2♂, 4♀) focal hepatic degeneration, and no single cell necrosis or increased incidence of centrilobular fibrocytes in males or females, minimal (1♂, 1♀) amounts of Kupffer cell and macrophage aggregates, minimal amounts (1♀) of inflammatory cell infiltration around central veins, minimal (2♂, 2♀) or zero amounts of pigmented Kupffer cells. There was minimal (1♂), trace (2♀), or zero amounts of pigmented macrophages in spleens of males and females in this dose group.

Controls – For controls (Table 7), there were minimal (2♀) or trace (1♂, 1♀) amounts of focal hepatic degeneration in the liver, minimal amounts of inflammatory cell infiltration in the portal area of the liver (1♂), and minimal amounts of pigmented Kupffer cells (3♂, 2♀). The incidence of pigmented Kupffer cells was greater in controls than in treated animals. There were no single cell necrosis and no aggregates of pigmented Kupffer cells and macrophages. There were minimal (1♂, 1♀) or trace (1♀) amounts of pigmented macrophages found in spleen.

2) Neoplastic – The study authors did not specifically state that they looked for neoplastic changes. There were no neoplastic changes recorded in the microscopic pathology report, MRID No. 428239-01

8. Neurological examination

Examinations were performed prior to beginning treatment and during weeks 34 and 50 to assess general physical condition, behavior, gait, cranial nerve function, spinal reflexes and postural reactions.

Results – All responses were within the normal range and there was no evidence of neurological abnormality or deficit in any of the animals.

D. DISCUSSION

The high dose for the study was originally chosen as 700 ppm, based upon results from preliminary 4-week and 13-week (Appendix B) dietary toxicity studies and a previously published report. In the preliminary studies, it was determined that the MTD of Ziram for

APPENDIX

Urinalysis: With the exception of the 1 male that was euthanitized, urinalysis parameters were within normal range for the remainder of the animals in the study.

Organ Weight Gain:

Liver: Group mean adjusted weights for liver for males and females at 1000 ppm were statistically significantly ($p < 0.05$) higher than control values.

Lungs and Heart: Statistically significant decreases in group mean adjusted heart weight for males at 1000 ppm ($p < 0.05$) and in group mean adjusted lung weights for females at 1000 ppm ($p < 0.01$) were considered by the study authors to be unrelated to treatment.

Macroscopic and Microscopic Pathology:

The liver from 1 female (1000 ppm) had "multiple depressed pale areas on the liver, focal necrosis, loss of cells and dilated sinusoids (note: biochemical findings for this animal at week 6 included increased bilirubin, AP, GPT, GOT, OCT, and cholesterol, however these levels were "unremarkable at week 13"). The liver from one male (300 ppm) also had focal necrosis. "Minimal amounts of pigment" in Kupffer cells from 2 males and 1 female (the female with liver necrosis described above) from the 1000 ppm group, and in Kupffer cells from 1 male and 1 female in the 300 ppm group. These changes were not seen in control dogs or dogs receiving 100 ppm.

Conclusions:

The toxicity data for the 13-week feeding study was used to set the highest dose level for the 52-week chronic dietary study at < 1000 ppm-essentially based on the finding of convulsive episodes for 1 male during week 5 (1000 ppm group). Other findings supporting the chosen dose levels were the biochemical, hematologic, and liver weight data. The lack of findings for dogs in the 100 ppm group was used as evidence for < 100 ppm as the NOAEL. On the basis of this study, levels of Ziram at 50, 185, and 700 ppm were chosen for use in the 52-week chronic feeding-dog study (MRID No. 428239-01).

Core Classification: Not applicable; dose range-finding study

There was a dose-related decrease in group mean body weight gain for females that was significant for the mid and high dose groups. Changes in clinical chemistry parameters included decreases in albumin (males and females) and total protein levels (females) and increases in SGPT (males) and alkaline phosphatase (males). Absolute weights for the liver were significantly increased for males in the high dose group. Significant dose-related decreases in absolute weight of the ovaries were observed in all dose groups. In males, there were microscopic pathologic findings (e.g., foci of degenerate hepatocytes, inflammatory cell infiltrations, and aggregates of macrophages and pigmented Kupffer cells) in the liver and pigmented macrophages in the spleen in the mid and high dose group.

IARC MONOGRAPHS
ON THE
EVALUATION OF THE CARCINOGENIC RISK
OF CHEMICALS TO MAN:

Some Carbamates, Thiocarbamates and Carbazides

Volume 12

This publication represents the views of an
IARC Working Group on the
Evaluation of the Carcinogenic Risk of Chemicals to Man
which met in Lyon,
9-15 June 1976

EJEM

1. Chemical and Physical Data

1.1 Synonyms and trade names

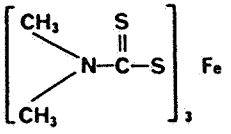
Chem. Abstr. Reg. Serial No.: 14484-64-1

Chem. Abstr. Name: Tris(dimethylcarbamodithioato-*S*,*S*')iron

Dimethylcarbamodithioic acid, iron complex; dimethylcarbamodithioic acid, iron (3+) salt; dimethyldithiocarbamic acid, iron salt; dimethyldithiocarbamic acid, iron (3+) salt; ferric dimethyldithiocarbamate; iron dimethyldithiocarbamate; tris(dimethyldithiocarbamato)-iron; tris(*N*,*N*-dimethyldithiocarbamato) iron (III)

Aafertis; Bercema Fertam 50; Ferbam 50; Ferbam, iron salt; Ferbeck; Fermate; Fermate Ferbam fungicide; Ferradow; Fuklasin Ultra; Hexaferb; Karbam Black; Stauffer ferbam; Sup'r Flo Ferbam Flowable; Trifungol; Vancide FE-95

1.2 Chemical formula and molecular weight



$C_9H_{18}FeN_3S_6$ Mol. wt: 416.5

1.3 Chemical and physical properties of the substance

From Stecher (1968), unless otherwise specified

(a) Description: Black solid

(b) Melting-point: Above 180°C (decomposition)

(c) Solubility: Soluble in water (120 mg/l at room temperature), acetone, chloroform, pyridine and acetonitrile

1.4 Technical products and impurities

Ferbam is available in the US as dusts containing 0.6-25% of the chemical, as wettable powders containing 3-98% and as a flowable formulation containing 42%. In Japan, ferbam is available as a technical product containing at least 95% of the chemical (Japanese Ministry of Agriculture & Forestry, 1975).

2. Production, Use, Occurrence and Analysis

For important background information on this section, see preamble, p. 15.

2.1 Production and use

Ferbam was first prepared and evaluated as a fungicide in about 1931 (Tisdale & Williams, 1934). It can be prepared by the reaction of dimethylamine with carbon disulphide in the presence of sodium hydroxide followed by addition of a ferric salt, such as ferric chloride (Kent, 1974). This is believed to be the method used in the commercial production of ferbam.

Ferbam has been produced commercially in the US since 1945 (US Tariff Commission, 1947). US production reached a maximum level of 1.4 million kg in 1961 (US Tariff Commission, 1962); in 1968, the last year for which production quantities were reported, 0.8 million kg ferbam were produced (US Department of Agriculture, 1972); in recent years, only 1 or 2 companies have produced it. Total US exports of all dithiocarbamate formulations were 6.7 million kg in 1974 (US Department of Commerce, 1975).

Ferbam is produced by one company in The Netherlands (Berg, 1975); one producer in France and one in the UK have an estimated total annual production of less than 1 million kg.

One company in Japan began producing ferbam in 1970. The quantity produced has decreased from 28.6 thousand kg in 1970 to only 700 kg in 1974 (Japanese Ministry of Agriculture & Forestry, 1975).

In the US, ferbam is used as a fungicide to control diseases of plants, particularly those affecting apples and tobacco (Berg, 1975), and is registered for use on about 75 agricultural and ornamental plants (US Environ-

mental Protection Agency, 1973). In 1971, 356 thousand kg were used on agricultural crops in the US (US Department of Agriculture, 1974).

In Europe, ferbam is utilized as follows: fungicide (80%), rubber accelerator (19%) and plastics pro-degradant (1%). In Japan, it is used on apples and citrus fruits (Japanese Ministry of Agriculture & Forestry, 1975).

A residue tolerance of 7 mg/kg has been established in the US for about 60 raw agricultural commodities (US Code of Federal Regulations, 1974), and many US commodities may bear residues of ferbam approaching this level. According to the US Occupational Safety and Health Administration health standards for air contaminants, an employee's exposure to ferbam should not exceed 15 mg/m^3 in the workplace air during any eight-hour workshift for a forty-hour week (US Code of Federal Regulations, 1975).

In December 1974, the Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues established a revised temporary acceptable daily intake for man of 0-0.005 mg/kg bw for all dithiocarbamate fungicides (WHO, 1975a,b).

2.2 Occurrence

Ferbam is not known to occur as a natural product. For information on the levels of ethylenethiourea, a possible breakdown product, that may be found on raw agricultural products, see IARC (1974).

As part of the 'Total Diet Program' of the US Food and Drug Administration, 360 composite food samples were collected annually during the period 1964-1970, prepared as for consumption and analysed for dithiocarbamate content. A maximum of 4 composites contained detectable levels of dithiocarbamates in any single year (Corneliussen, 1972), and in at least one year no dithiocarbamate was detected. On the basis of these results, analysis for dithiocarbamates in this programme was discontinued after 1970 (Manske & Corneliussen, 1974).

2.3 Analysis

Methods for the chromatographic analysis of carbamates, including dithiocarbamate pesticides, have been reviewed (Fishbein & Zielinski, 1967).

Ferbam residues have been determined by polarography, with a sensitivity of 80 µg/ml (Supin *et al.*, 1973) or of 10^{-7} M (Budnikov *et al.*, 1974). The voltametric behaviour of ferbam has also been studied (Golding *et al.*, 1974) and might provide the basis for an analytical method. A rapid colorimetric method for the estimation of ferbam residues on grains allows recoveries of 91-100% at levels of 10-1000 µg (Rangaswamy *et al.*, 1970).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Man

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: Groups of 18 male and 18 female (C57BL/6xC3H/Anf)F_1 mice and 18 male and 18 female (C57BL/6xAKR)F_1 mice received commercial ferbam (97% pure) according to the following schedule: 10 mg/kg bw in gelatine at 7 days of age by stomach tube and the same amount (not adjusted for increasing body weight) daily up to 4 weeks of age; subsequently, the mice were given 32 mg ferbam per kg of diet. The dose given was the maximum tolerated dose for infant and young mice but not necessarily so for adults. The experiment was terminated when the animals were about 78 weeks of age, at which time 16, 16, 16 and 15 mice in the four groups, respectively, were still alive. Tumour incidences were compared with those observed among 79-90 necropsied mice of each sex and strain, which either had been untreated or had received gelatine only: the incidences were not significantly greater ($P>0.05$) for any tumour type in any sex-strain subgroup or in the combined sexes of either strain (Innes *et al.*, 1969; NTIS, 1968).

Rat: Four groups of 25 4-week old rats of each sex were fed 0, 25, 250 or 2500 mg ferbam per kg of diet for 2 years. Controls lived for 600-700 days; median length of survival among rats given either 25 or 250 mg ferbam per kg of diet was not altered, whereas at the highest dose lifespan was about 430 days. There were 12 tumours in treated animals, and 7 in control rats; these were reported to be unrelated to dose. Tumour sites were not indicated (Hodge *et al.*, 1956).

(b) Subcutaneous administration

Mouse: Groups of 18 male and 18 female (C57BL/6xC3H/Anf)F_1 mice and 18 male and 18 female (C57BL/6xAKR)F_1 mice were given single s.c. injections of 100 mg/kg bw commercial ferbam (95% pure) in 0.5% gelatine at 28 days of age and were observed until they were about 78 weeks of age, at which time 15, 17, 18 and 16 mice in the four groups, respectively, were still alive. Tumour incidences were compared with those in groups of 141, 154, 161 and 157 untreated or vehicle-injected controls that were necropsied. Incidences were not increased ($P>0.05$) for any tumour type in any sex-strain subgroup or in the combined sexes of either strain (NTIS, 1968) [The Working Group noted that a negative result obtained with a single subcutaneous injection may not be an adequate basis for discounting carcinogenicity].

3.2 Other relevant biological data

(a) Experimental systems

In short- and long-term feeding studies in rats given diets containing 25-2500 mg/kg ferbam, apart from non-neoplastic brain changes in rats given the highest dose, no adverse effects were seen (Hodge *et al.*, 1952, 1956).

Approximately 40-70% of an oral dose of ferbam was absorbed from the gastrointestinal tract of rats during a 24-hour period. In rats that received ^{35}S-ferbam, 18% of the ^{35}S was excreted in the exhaled air as carbon disulphide and 23% in the urine. In those given [dimethyl-^{14}C]-ferbam, 43% of the radioactivity was found in the urine as dimethylamine and dimethyldithiocarbamate glucuronide (Hodgson *et al.*, 1975).

The combination of ferbam with ethanol caused accumulation of acetaldehyde in the blood (van Logten, 1972). Ferbam reacts with nitrite to form *N*-nitrosodimethylamine (Sen *et al.*, 1974).

When pregnant rats were dosed with [dimethyl-^{14}C]-ferbam, a small amount of radioactivity crossed the placenta and accumulated in the foetuses. Radioactivity was also secreted into the milk of lactating rats given [dimethyl-^{14}C]-ferbam and was in turn excreted in the urine of the pups (Hodgson *et al.*, 1974, 1975).

Administration of 150 mg/kg bw/day ferbam to pregnant rats on days 6-15 of gestation caused some foetal deaths, increased resorption, decreased foetal weight and produced a slight increase in the number of animals with soft and skeletal tissue abnormalities (Minor *et al.*, 1974).

Prasad & Pramer (1968) reported colour mutants and reverse mutations in *Aspergillus niger*; metabolic activation systems were not used in these tests.

(b) Man

No data were available to the Working Group, but for a discussion of the interaction of compounds such as ferbam with ethanol in the blood, see 'General Remarks on Carbamates, Thiocarbamates and Carbazides', pp. 28-29.

3.3 Case reports and epidemiological studies

No data were available to the Working Group.

4. Comments on Data Reported and Evaluation

4.1 Animal data

Ferbam has been tested by oral administration in mice and rats and by single subcutaneous injection in mice. Although no carcinogenic effect was observed in these tests, the available data are insufficient for an evaluation of the carcinogenicity of this compound to be made.

Ferbam can react with nitrite under mildly acid conditions, simulating those in the human stomach, to form *N*-nitrosodimethylamine, which has been shown to be carcinogenic in seven animal species (IARC, 1972).

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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